2ND RESTRUCTURED EDITION

PLANT BREEDING

THEORY & PRACTICES

V.L.CHOPRA

2nd Restructured Edition

PLANT BREEDING

Theory and Practices

Editor

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1

Genetic Basis of Plant Breeding

V. Arunachalam

Plant breeding is essentially a technology. Any technology will stand the test of time if it is based on sound concepts. In the case of plant breeding, the concepts are provided by the science of genetics.

Plant productivity in its broad sense requires consideration of not only yield but also of a number of its direct and indirect components. For example in groundnut, besides pod yield, shelling percentage, 100-kernel weight and oil content are components relevant to defining productivity. A number of physiological (like biomass, leaf area, specific leaf weight), morphological (like days to flowering, number of branches, number of mature seeds), nitrogen-fixing, (where relevant, for example, number of nodules, nodule mass, nitrogenase activity) and such other characters influence yield directly or indirectly.

Yield and most yield contributing characters are quantitative in nature. A quantitative character is one which shows continuous variation. In contrast, qualitative characters like flower colour and seed coat colour show, in general, discrete variation and are controlled by fewer genes with major effects. A quantitative trait, however, is governed by many genes. The range of values of a quantitative character is described by a continuous distribution, usually assumed to be a normal distribution. The distribution is specified by the two parameters: mean and variance.

The Basic Model

The fundamental model relates to the genetic value — G, of a genotype with its phenotypic value — P, expressed in its growing environment. The genetic value is a measure of inherent potential of a genotype which cannot be assessed exactly. To a large extent, differences in genetic values can be judged by differences in phenotypic values particularly when the environment affects the phenotypes uniformly in the same direction. For example, let three geno-types

A, B, C when grown during the normal rainy season have the genetic values 90, 95, 100 for days to maturity. When the genotypes are grown in post-rainy season under low temperatures during initial growth, let us say they have the phenotypic values 110, 120 and 130. The environment has prolonged the time to maturity in all the three genotypes. The relative order of days to maturity of A, B, C in the post-rainy season remains, however, the same as in the rainy season. Thus the basic genetic model can be written as

$$P = G + E \qquad \text{(i)}$$

where E is the contribution due to environment. The value of E can be positive or negative. Environment can be the entire growing environment which includes soil types and agro-climatic conditions like temperature, number of rainy days, pattern and amounts of rainfall. Locations or seasons in which all these factors vary are the commonly used environments in plant breeding.

In model (i), it is tacitly assumed that environmental contribution is independent of the genotype. In other words, the relative order of genotypes is assumed to remain the same in various environments. In practice however, some genotypes would be more adapted to specific environments (resulting in better phenotypic expression) than others and hence would have relatively high phenotypic values. For example, some varieties of wheat which are adapted to the North Indian relatively cold season and give high yields, perform poorly when grown in South India. Thus, genotypes do interact with environment and model (i) is modified for generalised application by including genotype × environment interaction also as:

$$P = G + E + (GE) \qquad \text{(ii)}$$

Genotype × environment interaction is important in plant breeding and merits special treatment. For a detailed treatment refer to Mather and Jinks (1971) and Hallauer and Miranda (1981). As a first level of approximation, model (i) only will be used in further discussion in this chapter.

Values, mean and variance

For quantitative characters like number of tillers and plant height, the phenotypic values, P,of genotypes can only be measured. None of the two variables, genetic value G and environmental contribution (or value) E, can be measured. Since the values of P, G,$E$are assumed to be from a continuous distribution, a relationship between variances of P, G and E from model (i) can be obtained as:

$$V_P = V_G + V_E \qquad \text{(iii)}$$

where G and E are assumed to be independent. From the observed values, V_P can be estimated. But both V_G and V_E cannot be calculated since the values of G or E are *a priori* unknown.

Hence an alternative is sought through the well set theory of designs of experiments due to R. A. Fisher. If the material under test is grown in a field design like randomised blocks design (r.b.d.), an estimate of environmental (or in the theory of designs, error) variance can be obtained. As an example, the ANOVA for 10 varieties (fixed genotypes) of wheat grown in a r.b.d. with five replications is given in Table 1.

Table 1. ANOVA of a r.b.d.

Source	d.f	mean squares	E (mean squares)
Replications	4		
Varieties	9	ms_1	$V_G + 5\,V_E$
Error	36	ms_2	V_E

Equating the expected values (obtained from the theory of r.b.d.) to the observed values of mean squares, the values of V_G and V_E can be estimated as:

$V_E = ms_2$;

$V_G = (ms_1 - ms_2)/5$

The following observations merit particular attention:

1. There should be no genetic variation within a variety so that plants of the variety occurring in various replications are genetically identical; segregating genotypes from F_2 generation, for example, cannot be replicated.
2. When requirements of conditions in (1) above are met, variation in a variety for the character under measurement between replications gives a measure of the environmental variation.
3. This experiment can also be extended over locations and seasons to take into adequate account the variation due to $G \times X \times E$ interactions, in its broad sense.

Having experimentally evaluated V_G, one should understand, using basic principles of genetics, how it is related to the genetic values, G. As mentioned earlier, G cannot be measured. For easy comprehension, let us assume that a quantitative character X is controlled by a single gene with two alleles, B, b. There will then be three genotypes, BB, Bb and bb.

Let the genetic value of the recessive genotype, bb be y. The genotype Bb can be obtained from bb by substituting one dominant allele B for the recessive

allele, b. Similarly the genotype BB can be obtained from Bb by a single gene-substitution or from bb by two gene-substitutions. Fisher (1941) defined the genetic value added by such a gene substitution by a quantity, α called the 'average effect of gene substitution.' The genetic values and frequencies of genotypes can then be written as:

Genotype	Frequency	Genetic value G
BB	p^2	$y + 2\alpha$
Bb	$2pq$	$y + \alpha$
bb	q^2	y

where p = frequency of gene B, $q = 1 - p$ = frequency of gene b.

A look at the G values above will indicate that they are additive in scale. In other words, from the value of bb, the value of Bb is obtained by addition of ?and of BB, by addition of 2 α. The values y, $y + \alpha$, $y + 2\alpha$ will always lie on a straight line. Thus the values y, $y + \alpha$, $y + 2\alpha$ for the genotypes bb, Bb and BB are called '*Additive values*'and are denoted by A.

It is not necessary that genetic values should necessarily be additive in scale, as explained above. They may have, in principle, any value x_1, $x_2$and x_3 for bb, Bb and BB respectively. They will then deviate from the additive values, the deviations being *(G–A)*. Such deviations occur as a result of dominance and hence are called dominance deviations. They are denoted by D.

Thus genetic values G, in principle, are made up of two components –A additive value and D,dominance deviation. So that

$$G = A + D \qquad \text{(iv)}$$

Further, A and D are independent (and hence uncorrelated), so that the variance of genetic values, V_G (or total genetic variance) is given by

$$V_G = V_A + V_D \qquad \text{(v)}$$

Where V_A is the additive genetic variance, that is variance of additive values and V_D is the dominance variance, that is variance of dominance deviations.

Usually a linear regression (straight line) is fitted to the genetic values, $G x_1$, x_2, x_3 by least squares technique. This 'best' straight line is the one that passes through the three additive values, A. The best estimates of the parameters α and y are obtained through the least squares method as:

$\alpha = p(x_3 - x_2) + q(x_2 - x_1)$

$y = \bar{x} - 2p\alpha$ where = mean of genetic values, G
(that is x_1, x_2, x_3)

$V_A = 2pq\alpha^2$ and

$V_D = 4p^2 q^2 h^2$ where $2h = x_3 - 2x_2 + x_1$ (vi)

α, the additive effect is a very important parameter in the theory of plant breeding. In fact, the breeding value of an individual (genotype) is a function of α.More specifically, in our example, the breeding values of BB, Bb and bb are given by $2q\alpha$, $(q-p)\alpha$ and $-2p\alpha$ respectively. Falconer (1981) has defined the breeding value of a genotype as equal to twice the deviation of its progeny mean from the population mean, when the genotype mates at random with other members of the population. In fact, it is also the general combining ability effect of the genotype. It may be noted that this definition is valid only for genotypes governed by a single gene.

The points described so far will be better understood through a numerical example. In a population, let there be 25 per cent of each homozygote and 50 per cent heterozygotes, that is the genotypes, bb, Bb and BB occur with frequencies 1/4, 1/2, and 1/4. Then the gene frequency of gene B, $p = 1/2$ and of b, q = 1/2. Let the genetic values of a quantitative character for these genotypes be 10, 20 and 18 respectively. Then

$\alpha = 1/2\,(18 - 20) + 1/2\,(20 - 10) = 4$
$\bar{x} = 1/4\,(10) + 1/2\,(20) + 1/4\,(18) = 17$
$y = 17 - 2.1/2.4 = 13$
$2h = 18 - (2 \times 20) + 10 = -12$

Genotype	Frequency	Genetic value G	Additive value A	Dominance deviation D=G–A
BB	1/4	$x_1 = 18$	$y + 2\alpha = 21$	–3
Bb	1/2	$x_2 = 20$	$y + \alpha = 17$	3
bb	1/4	$x_3 = 10$	$y = 13$	–3

Now, V_G = Variance $(x_1, x_2, x_3) = 17$
V_A = Variance of $(y, y + \alpha, y + 2\alpha) = 8$
V_D = Variance of dominance deviations, that is of $(-3, 3, -3) = 9$
Otherwise also, $V_A = 2.1/2.1/2.4^2 = 8$ and
$V_D = 4.1/4.1/4.36 = 9$ from the formulae given in (vi)

Thus, it is obvious that $V_G = V_A + V_D$, that is the total genetic variance is the sum of additive genetic (V_A)and dominance (V_D)variances, as observed earlier. Then it follows from the equation (iii), that the phenotypic variance

$$V_P = V_G + V_E = V_A + V_D + V_E \quad \text{(vii)}$$

When a two-gene model (with no linkage) is considered, not only A and D components at each locus, but also their interactions are relevant. Denoting A and D components for the two genes as $A1$, $D1$, $A2$,$D2$respectively, the genetic variance will be given as

$$V_G = V_{A1} + V_{A2} + V_{D1} + V_{D2} + V_{A\times A} + V_{A\times D} + V_{D\times D} \quad \text{(viii)}$$

Suppose, for example, the two genes are H and K. Let the marginal genetic values of HH, Hh and hh (when the effects are pooled over the other gene (K) be 7, 8, 3 and similarly that of KK, Kk and kk (pooled over the geneH) be 8, 6, 2 respectively. If the genetic values of the two-locus genotypes are obtained purely in an additive manner, the values will be as follows:

		KK 8	KK 6	kk 2
HH	7	15	13	9
Hh	8	16	14	10
hh	3	11	9	5

In this case, there will be additive and dominance variances at each locus but there will be no interaction effects. In other words, the value of HHKK genotype is the total of the values of HH and KK genotypes only; the value of HHKK does not get enhanced or reduced when HH and KK come together. If on the other hand, the value of HHKK = 18 units, there is an interaction effect =18 – 15 = 3 units. Similar observations hold good for other genotypes. When there is no interaction,

$$V_G = V_{A1} + V_{A_2} + V_{D1} + V_{D2}$$

In practice, the absence of interactions is rare. It may, however, be noted that interactions are relevant only when a character is controlled by more than one gene. The intergenic interaction variance, to describe it fully, will then be made up of additive × additive, additive × dominance and dominance × dominance interaction variances. These interactions are jointly known as epistatic interaction. Different types of interactions are dealt with in detail along with examples in Crow and Kimura (1970); Mather and Jinks (1971) and a fuller treatment of genetic variances in one or two genes in the above references and also in Cockerham (1954); Kempthorne (1957); Arunachalam and Owen (1971); and Falconer (1981).

Additive genetic variance, V_A is important for the following major reasons:

1) The proportion of additive to phenotypic variance for a character estimates its narrow sense 'heritability' denoted as h^2, and the response to selection is directly proportional to h^2 (Falconer 1981). Additive genetic variance gets depleted proportionate to the improvement made by selection.
2) Breeding value of an individual is measured directly by the additive effect. Though this has strictly been proved for single gene governed characters, the applications of this concept hold general validity. The general combining ability (gca) effect of a parent is a function of breeding value and hence of the additive effect.

Covariance between relatives

Covariance and correlation between relatives are important concepts in plant breeding. Progress by selection is directly proportional to the degree of resemblance between the parent and its progeny. The genetic constitution of the selected parents and the mating system employed to produce the progeny will determine the genetic constitution of the progeny population. Thus, in a simple instance, of two parents, BB and bb, the progeny will contain all the three genotypes, BB, Bb and bb when the parents are intermated (since all the matings of the type BB × BB, BB × bb and bb × bb are possible) but will consist of the genotypes, BB and bb only when they are selfed.

Covariance between relatives is expressed as a function of additive genetic variance and hence provides an alternative method of estimating heritability. This assumes importance while estimating additive variance. In comparison with estimation of a mean, estimation of variances suffers from inaccuracies; violent variations in individual values affect it still further. As a result, components of variance sometime turn out to be negative.

Heritability is estimated, however, as twice the regression coefficient of parent on offspring. This estimation uses the covariance between parent and offspring. Such regressions are not free from problems but they do, at least,avoid the troubles associated with partition of variance. In general, they offer a more secure approach to estimating heritability (Gilbert, 1973; Simmonds, 1981).

Of the many relatives, parent-offspring, full- and half-sibs are commonly used in plant breeding contexts. While the offspring of a cross between two known parents are related as full-sibs, the offspring of two crosses in which only one parent is common are related as half-sibs. As an example, consider a three-parent diallel cross whose parents are designated as K, L, M. If the F_1 is grown in a randomised blocks design in two replications, plants of a cross like K × L in any one or both of the replications are full-sibs; similarl yplants of the crosses,

K × L and K × M, are related as half-sibs. In plants, full-sibs are generated by controlled pollination, that is by conscious choice of pollen parents. For producing half-sibs, only female parents are identified.Usually, production of half-sibs is easy in cross-pollinated crops like maize.It must be understood that when open-pollination is the process by which half-sibs are produced, half-sibs may result from pollen from more than one genotype. As a result, half-sibs from crosses with multiple pollen would be genetically more heterogeneous than those from crosses with pollen of a single genotype. Since these two cases can hardly be distinguished in practice, decisions based on the performance of half-sibs without consideration of pollen source may become imprecise.

Additive genetic variance can be estimated from any of the following co-variances:

$$\left.\begin{array}{lll} \text{Cov (parent, offspring)} & = & 1/2V_A \\ \text{Cov (full-sibs)} & = & \tfrac{1}{2} V_A + \tfrac{1}{4} V_D \\ \text{Cov (half-sibs)} & = & \tfrac{1}{4} V_A \end{array}\right\} \quad \text{(ix)}$$

(Falconer, 1981)

Important mating designs

Of the various designs, random mating is fundamental. Here, an equal chance is provided for every possible mating in a population; where relevant, such matings include selfing also. In some mating designs, only specific matings are permitted in accordance with requirements of the design. For instance, let us assume that a single cross produces the progeny genotypes, QQ, Qq and qq. They are full-sibs. If the next generation is produced by full-sib mating, all the possible matings among the three genotypes can occur. But if it is produced by selfing, only the three matings, QQ × QQ; Qq × Qq and qq × qq are possible. Consequently, the nature and frequency of the resulting progeny genotypes under the two mating designs will be different.

Mating designs employed in plant breeding are essentially decided by the pollinating system. While selfing is the usual method of mating adopted in self-pollinated crops, intermating (full-sib mating) is used in cross-pollinated crops. It is essential to understand the changes in genetic structure brought about by continued selfing or intermating over generations. Consider the F_1 of the single cross, QQ × qq. The F_1 generation will be genetically uniform and consist of the heterozygote, Qq only. Under selfing and intermating the frequency of heterozygotes will change in each generation. If the frequency of heterozygotes is h in a given generation, homozygotes QQ and qq will occur with equal frequency – $(1 - h)/2$at that generation. The change in the frequency of heterozygotes in the first 10 generations is given below for the two mating systems:

Generation										
Frequency of heterozygete	Selfing	1	2	3	4	5	67	89	10	
	Full-sib	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256 1/512
	Gotemating	1	1/2	2/4	3/8	5/16	8/32	13/64	21/128	34/256 55/512

It can easily be seen that selfing rapidly reduces heterozygosity and leads the population to homozygosity. But full-sib mating reduces heterozygosity at a much lower speed. In fact while selfing retains only 0.2 per cent of heterozygotes at generation 10, full-sib mating retains 56 times the frequency retained by selfing. Further, heterozygotes which are "balanced" genotypes, are better buffered against various stresses and possess homeostatic properties. Retention of high frequency of heterozygotes will therefore help in maintaining genetic variability for selection. This is the reason why efficient plant breeding places a high premium on intermating, such as biparental mating (equivalent to full-sib mating in F_2 generation), for example.

Diallel crosses and line × tester mating are two designs often used in plant breeding. While in a diallel cross, each genotype is used both as a female and a male parent, in a line × tester system, different sets of male and female parents are used. The crosses from these mating designs are evaluated in F_1 generation and desirable parents or crosses are selected on their combining ability components.

General combining ability (gca) is defined as the average ability of a parent to combine with a set of other parents in crosses. Gca effect of a parent, K, is therefore computed as $(G - M)$ where G is the mean value of crosses in which K is a parent and M,the experimental mean. It is known from elementary principles that G is the mean value of half-sibs for the character under measurement. Hence, in the simplest case variance of gca, denoted as var (gca), is given by Cov (half-sibs) $=4/2\ V_A$. In general, Var (gca) contains additive variances and additive type of interactions.

Specific combining ability (gca) is relevant to a cross and is defined as its specific ability to produce a high mean value. The high mean value is attributed to favourable interaction effects when two parents come together in a specific cross combination. The sca effect of a cross is calculated as $(S - M - G1 - G2)$ where S is the mean value of the cross; M, the experimental mean; and Gl, G2 the gca effects of the two parents involved in the cross. Analogous to Var (gca), variance of sca, denoted as Var (sca), is given by Cov (full-sibs) – 2 cov (half-sibs). Using equations (ix) given earlier, in the simple case of a single gene system,

$$\text{Var (sca)} = 1/2\ V_A + 1/4\ V_D - 2.1/4\ V_A = 1/4\ V_D$$

Thus Var (sca), in the general case, contains dominance variance and dominance-based interactions.

Estimation of Var (gca) and Var (sca) by evaluation of crosses made to a design in their F_1 generation is thus a more efficient method to understand the type of gene action controlling the inheritance of a quantitative trait. It is pertinent to note that an estimate of environmental variance is obtained by ANOVA based on the field design. The functions of additive and dominance variances and epistatic interactions are estimated by Var (gca) and Var (sca). Though the method of approach is different, the basic principles are the same as explained in section 1.1.

Heterosis

Though breeders sometime make a choice of parents based on visual examination of desirable attributes sought, a more rational approach is to base the selection on combining ability effects and F_1 heterosis. In principle, a choice based on estimable parameters relevant to gene action should be preferred as a time and resource efficient strategy.

Heterosis is the superiority of a F_1 hybrid over its parents. When this information is to be used for achieving improvement in the character under measurement (like yield, maturity and so on), it is appropriate to calculate heterosis over the better (of the two) parent. Further it is necessary to check whether the hybrid mean is statistically superior to the better parent mean, as numerical superiority need not always be decisive. Only when the hybrid superiority is statistically significant heterosis is calculated as the per cent superiority of the hybrid in the desired direction over the better parent. In all other cases, heterosis can be taken to be absent. An example will illustrate the point further.

	Days to maturity X	Number of Branches Y	Yield (q/ha) Z
Parent 1	110	30	20
Parent 2	95	25	18
Hybrid	100	32	25
t-test of (hybrid mean –better parent mean)	Significant	Not significant	Significant

In the case of the character X, the hybrid matures later than the earlier maturing 'parent 2'; in the context of breeding for early maturity therefore, there is no heterosis for X. It may be noted that a negative value for heterosis is not calculated in this case, as it permits no useful interpretation. In the case of Y, though the

hybrid has two branches more than the better 'parent 1,' there is no heterosis as the difference is not statistically significant. In the case of Z, heterosis is observed for yield and is computed as; (25–20)/20 × 100 = 25 per cent. The above criteria, though are not mandatory, help decision making simple and efficient.

Combining ability and heterosis

A comparison of the relative magnitudes of the estimates of Var (gca) and Var (sca) will help to identify the predominant type of gene action governing the metric trait. Two situations in this context can be clearly distinguished:

a) In the ANOVA of combining ability, variation due to gca significant by F-test and that due to sca not significant; estimated Var (gca) higher thanVar (sca).

This will imply that additive gene action is predominant and hence direct selection for higher values of the character can be made in further generations of the F_1 material. The choice of specific crosses, in which a search for transgressive segregants possessing higher values for the character can preferentially be made, will require a further study of the magnitudes of gca effects of parents, sca effect and F_1 heterosis for the character.

b) In the ANOVA, variation due to gca non-significant by F-test and that due to sca is significant; estimated Var (sca) higher than Var (gca).

This situation indicates that dominance and epistatic interaction are predominant for the character. Further, if F_1 heterosis is very high for a specific cross and the observations made are true for an economic character like yield, it is possible to utilise the cross as a commercial hybrid. It is, however, important that the pollinating system of the crop should either permit commercial seed production of the hybrid or the female parent should be so chosen as to have cytoplasmic-genetic male sterility (with proper maintainer lines for producing seeds of the female parent on a commercial scale).

Selection

Selection is a major and important process in breeding for improvement of one or more plant attributes. As has been pointed out earlier, a prerequisite is the availability of additive genetic variance.

Selection is the process of retention of desired genotypes and elimination of undesirable ones. In the population under selection for a metric character, genotypic frequencies and hence gene frequencies are altered. These changes are further modified by the mating designs employed to advance the selected individuals to the next generation.

Selection is of two kinds—natural and artificial. The former, as the name implies, is carried out by nature while the latter is practiced by a breeder for traits preferred by him.

Natural selection

It manifests itself through differences in genotypic fitness. Fitness, as understood today, is a complex parameter which encompasses, among others, adaptation, survival ability and viability. A full exposition of fitness and related concepts can be found in Spiess (1977). In a broad sense, viability of an individual is measured by the number of its offspring that survive to maturity and produce at least one offspring each.

Usually, the viability of the heterozygote is taken to be unity and the relative viabilities of the homozygotes are expressed in relation to it. For example, if in comparison with 100 per cent viable offspring produced by the heterozygote Bb, the dominant homozygote BB produces only 80 per cent, and the recessive homozygote bb produces only 60 per cent of viable offspring, the viabilities of genotypes bb, Bb and BB are represented as 0.6, 1 and 0.8 respectively. In other words, there is a 40 per cent disadvantage to bb and 20 per cent to BB compared to Bb, in contributing offspring to the next generation. These disadvantages are known as selective disadvantages or selection coefficients. In this case, selection coefficient for BB, $s_1 = 0.2$ and selection coefficient for bb, $s_2 = 0.4$. The viabilities are then expressed as:

	BB	Bb	bb
Viability	0.8	1	0.6
=	$1 - S_1$	1	$1 - S_2$

The situation described above is commonly described as 'heterozygote advantage.'

The changes in genotypic frequencies in a population are brought about by two factors: (i) natural selection, and (ii) the mating design employed to forward the population from one generation to the next. When these two forces are in operation, the reduction or increase in gene frequencies occurring due to one force will be compensated for by the other. For example, if the frequency of the recessive genotype is reduced by lower relative viability (natural selection), it can increase due to random mating which brings in heterozygote × recessive homozygote mating (mating design). In this process, there soon comes a stage at which the gene and genotypic frequencies remain stationary; balanced action of the two forces can maintain this stationary state for a number of generations

under certain conditions. Such a stationary state is known as an equilibrium. Some of the equilibria are stable (over time) and some are not. Trivial states of stable equilibria are, for example, provided by populations under natural selection which have reached complete homozygosity (members of the population being all dominant or all recessive homozygotes). One other interesting stable equilibrium is one in which all genotypes are present. This equilibrium is called a balanced polymorphism. There are various situations in which such polymorphisms occur. A fuller treatment of the subject can be found in, for example, Li (1955); Spiess (1977); Crow and Kimura (1970); Arunachalam and Owen (1971) and Falconer (1981).

Artificial selection

This is selection made by a breeder for desired traits. Usually, a proportion of individuals possessing relatively high phenotypic values for the trait under consideration is selected from a base population and advanced to the next generation. The mean of the progeny population will then be higher than the mean of the base population. This difference is known as the response to selection. Response is directly proportional to the proportion of the base population selected and heritability of the trait. As in the case of natural selection, the design of mating to which the selected individuals are subjected will also influence response. A number of methods of artificial selection are available to a breeder to suit various objectives, pollinating system of the crop and the other factors that govern the ultimate end product yield. These are dealt with in excellent detail in some of the books suggested for further reading.

It must be noted that artificial selection is always superimposed on natural selection. Most often they work in opposite directions. For example, nature will select small seeds to aid perpetuation of species and maintenance of large variation; a breeder will select bold seeds. The artificial selection pressure has to be sufficiently strong for a breeder to succeed in breeding a variety with bold seeds. Such bold seeded varieties also require better growing environments providing for adequate irrigation, fertilisers and so on. For example, groundnut varieties with extra large kernels (hand picked seeds) need to be raised on good soil under frequent irrigation. Naturally, the maintenance of such varieties of different crops depends on the pollinating system, their genetic constitution and seed production methods in addition to the provision of proper growing environments.

Genetic Basis for Producing Cultivars

The genetic principles outlined so far will help to describe the various genetic forms in which the breeders produce material for maintaining productivity.

Pure line: Pure line is most often used synonymously with 'variety'. In the modern context, since the term 'variety' is used in a broader sense, the alternative term pure line should be preferred.

Initiating from a single/multiple cross, pure lines are derived after a few generations of pedigree breeding. They are homozygotes and represent stable but trivial equilibria. They are genetically and phenotypically homogeneous. They are relevant to self-pollinated crops. Seeds of pure lines can be produced on a commercial scale easily. In self-pollinated crops like wheat and rice, pure lines can simply be grown for seed production in large areas and seeds collected.

Pure lines are genetically vulnerable. Their productivity may be drastically hit by the occurrence of a disease, to which they are susceptible in epidemic proportions. For example, several pure lines of NP wheat, Adt rice and of other crops like barley, sorghum, linseed and groundnut have gone out of cultivation in India. If, in future, one such pure line that went out of cultivation needs to be derived *de novo,* theoretically it is not possible to regenerate it, as it would be impossible to replicate identically the process of pedigree breeding that was earlier used to derive it.

Hybrid

They are pure heterozygotes and hence are genetically and phenotypically homogeneous. They represent unstable equilibrium, since in the next generation they will segregate into homozygotes and heterozygotes.

Seeds of hybrids are hence to be produced every season. When seeds are required on a commercial scale, it will not be economical to produce them through manual operations of emasculation and pollination. Further, even in highly cross-pollinated crops like maize and pearl millet, maintaining the genetic purity of two parents over a long time can be arduous. For ease of commercial seed production, the female parent is selected/bred as a cytoplasmic-genetic male sterile line. It is maintained by crossing to a maintainer line. The male parent is maintained as an inbred by selfing, sib-mating or both.

Hybrids also can go out of cultivation. But if pure seeds of their parents are available, hybrids can be regenerated. Once a hybrid is unable to meet the requirements of high productivity, it is usually replaced by a better one.

Hybrids have better adaptability to environmental and other stresses due to internal balance as compared to pure lines. Their response to agronomic inputs like irrigation and fertilisers is high. They usually yield better than pure lines.

Population

It is a reproductive community of sexual and cross-fertilising individuals which share a common gene pool (Dobzhansky, 1951). By nature populations are relevant only to cross-pollinated crops.

Populations are polymorphisms containing homozygotes and heterozygotes. If they are balanced polymorphisms maintaining all genotypes, they can sustain their yield levels for a longer period. If, on the other hand, they contain only a few of the possible heterozygotes and homozygotes, some of them (component genotypes) can get eliminated in due course of time due to various causes like inadequate population size. As a result, the populations themselves can go out of existence. But balanced polymorphisms can afford protection against such hazards and can help to prolong the life of populations.

Populations possess a number of advantages:

1. They are stable and highly adaptable. They can withstand biotic and abiotic stresses much better than pure lines or hybrids, since they contain an array of genotypes, particularly, a number of highly adaptable heterozygotes.
2. Unlike hybrids, their seeds can be produced on a commercial scale with ease. The only requirement is that seeds should be produced on large plots under isolation. Farmers can be trained to produce and retain seeds for their own use so that the cost of seeds will be low and affordable.
3. Populations are dynamic. An array of genotypes is regenerated every generation by the large random mating in seed production plots. In a population, such genotypes function in a coadapted manner. They thus serve as a natural barrier against rapid evolution of new virulent races of pathogen.

However, populations are genetically heterogeneous. Their phenotypic homogeneity is much less compared to pure lines or hybrids. Hence separate seed certification standards, that allow for reasonable levels of heterogeneity for plant traits become necessary.

Seed production of populations should be done in isolated large plots so that large scale random mating and hence production of all genotypes in appropriate frequencies are possible. But, as mentioned earlier, slight changes in the frequency of genotypes can occur, since in practice, we cannot handle infinitely large populations. But the yield of a population is the aggregate yield of constituent genotypes. When the constituent genotypes change in their frequency, the yield of a population can also fluctuate. Usually this fluctuation is reasonably small. The range of fluctuation is narrow because the forces of selection restore the slightly deviating gene and genotypic frequencies to the stable equilibrium values. Thus, as against the fixed yield level of pure lines, only a range of yield (though a narrow range) can be set aside for populations.

Two types of population are in use—synthetic and composite.

The initial gene pool of a synthetic population is composed of a number of inbred lines which have earlier been selected to have desirable gca for various attributes. Inbred lines are used as components of initial gene pool for their easy maintenance. They are selected for gca so that, whatever be the inbreds that take part in intermating as parents, their progeny will be productive.

However the performance of a population can decline after a few years. Several reasons can be appended for such a decline. Two of them are:

a) Preferential elimination of particular genotypes by a disease (exerting a force similar to selection pressure) prevalent in the growing environments. This will cause a deviation in the genotypic and gene frequency. When the deviation is too high for selection to restore the population to polymorphic equilibrium, it can drift away from it and ultimately settle in a trivial equilibrium (complete homozygosity).

b) Due to inadequate size of population maintained in seed production plots, non-random mating (due to low frequency of matings or elimination of mating between some genotypes) may occur. This will upset the balance in the frequencies of progeny genotypes leading to a situation similar to that mentioned in (a).

In the case of a synthetic population, when such a decline in performance is noted, the initial gene pool can be made afresh using the inbred parents and theoretically the synthetic population can be reconstructed.

In contrast, a number of possibilities exist to construct the initial gene pool of a composite population. The components of the gene pool can be single plants derived from one or more single or multiple crosses, or from various germplasm source lines possessing desired attributes and so on. But all of them need to be first evaluated to ensure that they possess the desired attributes including yield. Since segregating genotypes can also be in the initial gene pool, there is no need or provision for maintaining the components of initial gene pool. Depending on the nature and extent of hetero-geneity in it, the number of intermating and selection cycles required to bring phenotypic homogeneity with productivity can be high.

When, for the reasons cited earlier, the productivity of a composite population declines, it cannot be reconstituted or regenerated. This is the major difference between synthetic and composite population. However, the declining composite population can easily be replaced by another in the breeders' pipeline. A factor compensating the lack of capability for reconstitution is the relative ease with which a new composite population can be derived starting from a fresh initial

gene pool. A fuller treatment of the concepts and breeding strategies can be found in Arunachalam (1980) and Arunachalam and Katiyar (1982).

Multilines

They are relevant to self-pollinated crops. They are mixtures of isogenic lines with desirable agronomic characters including yield. These lines can be bred for resistance to one or more races of pathogen. There are two types—clean crop and dirty crop—of multilines. In the former, each isogenic line has resistance to all known races of pathogen, while in the latter this is not insisted upon, but each one should be resistant to at least one race. Both the multilines have built-in insurance against disease risks.

Varietal blends

They are similar to multilines but each component, instead of being an isogenic line, is a different variety or pure line. As a blend they can stand environmental stresses better and ensure, to a certain extent, stability in yield. In crops like groundnut where biotypes of pathogens for diseases like rust and leafspot are not yet identified, varietal blends incorporating component varieties with stable field resistance and good yield, may perform better than a pure line.

The salient features of genetic forms in which productivity can be maintained are given in Table 2.

From the concepts of partitioning phenotypic value into genetic and environmental components, the idea of partitioning measurable phenotypic variance into genetic and environmental variance components was developed. Genetic variance was further partitioned into additive and dominance components, in the simple case (and epistatic interactions, in addition in other cases). Estimation of the components of genetic variance was then dealt with under various designs of mating. It was realised that improvement in the desired trait can be sought through selection provided sufficient additive genetic variance exists because response to selection is directly dependent on it. Efficient breeding strategies would then be expected to be based on a purposeful combination of mating systems and selection.

It is not possible to deal with every aspect mentioned above in detail; each one of them is so wide, that several books deal with them exhaustively (*see Suggested Readings*). Nevertheless, it is shown in this chapter that purposive plant breeding has, and needs to improve on, a firm genetic base.

Table 2: Salient features of genetic forms

*Genetic form	Relevant to	Genetic Constitution	Nature	Equilibrium	Re-constitution
Pure line	Self-pollinated crops	Single Homozygote	Homogeneous	Trivial; Stable	Not possible (in general)
Hybrid	Cross- and self) pollinated crops	Single Heterozygote	Homogeneous	Unstable	Possible
Composite population	Cross-pollinated crops	Homo- and Heterozygotes	Heterogeneous	Polymorphic Stable	Not possible
Synthetic population	Cross-pollinated crops	Homo- and Heterozygotes	Heterogeneous	Polymorphic Stable	Possible
Multilines	Self-pollinated crops	Several isogenic Homozygotes	Heterogeneous	Stable	Possible
Varietal blends	Self-pollinated crops	Several Homozygotes	Heterogeneous	Stable	Possible

*All are phenotypically homogeneous.

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2

Concepts and Methods: Self-Pollinated Crops

D. Sharma

Man-directed evolution of distinct types and cultivars (cultivated populations) of self-fertilised crops had their beginning about 10,000 to 12,000 years ago. As a breeding system, self-fertilisation favoured constancy and fitness (Stebbins, 1950) of the genotypes to the immediate environment. This provided an alternative to the slash and burn migrant agriculture of the Neolithic farmers (7,000-6,000 B.C.) and to stabilisation of production.

Manu Smriti (700–500 B.C.) proclaimed *Subeejam Sukshetre Jayate Sampadyate* meaning that good seed in a good field yields abundant produce. The knowledge that good seed is essential for a good harvest, led to conscious efforts for selecting types which provided a dependable source of food. Besides their utility as food, choice of plants and their seeds as progenitors of the next generation depended on personal fancy, observation and experience. As farmers learnt their crop plants better, they sorted them out into similar types (species). While migrating from the centres of origin they carried seeds of mixed populations.

Self-pollinated cereal crops of barley, wheat, oats and important grain legumes such as lentil, peas and chickpea spread from the Near East Centre, first to far and remote areas of Asia, Europe and Africa; and subsequently in comparatively recent times, to the Americas and Australia. In new environments, the introduced mixed populations diversified into well adapted identities of land races. These land races continued to be the main basis of cereal production till early 1900. Improvement in crop plants made by some early civilisations was mainly through introduction and mass selection, based on the natural tendency of preserving the seeds of preferred colour and size from vigorous, healthy and more productive plants.

It was only after the rediscovery of Mendel's laws in 1900 that breeding procedures based on the knowledge of the genetic principles and the inheritance

pattern of different characteristics were developed. This permitted the improvement of crops with objectivity and predictability.

Genetic Consequences of Self-Fertilisation

Self-pollination is the transfer of pollen from an anther to a stigma within the same flower or to a stigma of another flower on the same plant. In other words, formation of a seed by union of the male and the female gametes from the same plant is self-fertilisation or autogamy. Therefore, in a self-pollinated crop a parental genotype AA is perpetuated in its progeny, unless there is a chance cross-fertilisation with plants having the genotype aa. Some cross-pollination does occur in crops normally classified as self-pollinated. The degree of cross-pollination depends on: (1) the crop variety or strain; (2) seasonal conditions, particularly temperature and humidity; (3) wind direction and velocity at the time of pollination; and (4) population of pollinating insects. Based on the extent of cross-pollination occurring in the different species, crop plants are grouped into: (a) self-pollinated; (b) often cross-pollinated; and (c) cross-pollinated.

In terms of a breeding system, self-pollination is an extreme form of inbreeding, which not only perpetuates the parental genotypes from one generation to another but also rapidly restores homozygosity of the genotypes in generations following a chance cross-pollination (hybridisation) in nature or on artificial hybridisation. In a self-pollinated crop, the proportion of heterozygosity in subsequent generations of a hybrid Aa is reduced by one half in each generation for the locus concerned as is shown in Fig. 1. The rate of return to homozygosity in each generation can be calculated by the formula $(2^m - 1/2^m)^n$, where m is the number of generations and n is the number of heterozygous gene pairs. The formula assumes no selection (such as equal survival and reproductive rate of all genotypes and independent inheritance of all gene pairs). If heterozygotes have advantage over the homozygous genotypes, return to homozygosity is delayed and polymorphism (presence of heterozygotes as well as homozygotes for the alleles at a locus) persists in the population. Advantage to any of the homozygotes results in quick return to homozygosity and fixation of the favoured allele (A or a) in the population. Linkage increases the proportion of homozygous genotypes for one or the other allele of the linked genes but does not change the percentage of homozygous gene pairs that is percentage of homozygosis (Wright, 1921). As can be calculated from the formula, selfing for 5 to 10 generations will render most individuals of a breeding population homozygous for a number of gene pairs. Even with 100 independent gene pairs in a hybrid, 90 per cent of the individuals will be homozygous after selfing for 10 generations.

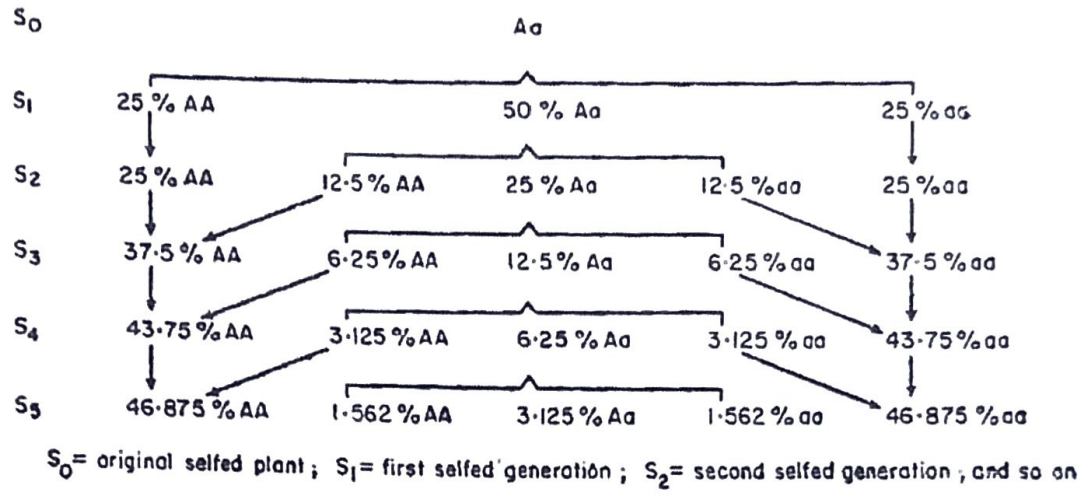


Fig. 1. Proportions of homozygous and heterozygous genotypes in a population after successive generations of self-pollination, assuming equal fitness for survival among genotypes.

However, this homozygosity at most loci does not mean homogeneity of the population with regard to the genotypes. Since, an heterozygote (Aa) at a loci gives rise to two homozygous genotypes (AA and aa), one each for the two alleles, the possible homozygous genotypes will be 2n, where n represents the number of heterozygous gene pairs. With only 20 gene pairs, the possible homozygous genotypes are 10,00,000. This clearly shows that land races of self-pollinated cereal and grain legume crops are mixture of a vast number of pure lines. The land races offered a great scope for adaptation and improvement of types by natural selection in new environments of migrating farmers and early plant breeders.

It was only in the late eighteenth century that conscious systematic selection of plants from a population was recognised as a plant breeding procedure. In 1843, Le Couteur was the first plant breeder to report his work and clearly indicated the importance of selecting individual plants for the improvement of cereal crops.

However, Louis de Vilmorin in France (1843–1890) selected the best plants in each generation of single plant progenies of four wheat varieties for 50 consecutive years, but did not detect an appreciable improvement. Similarly, the breeders of the Swedish Seed Association at Svalof (1886–1890) practiced mass selection by continuous selection of the biggest plants and the biggest heads in the variety Chevalier of barley with the objective of improving straw strength but did not realise any recognisable improvement. These observations indicated that selection of individual plants in an autogamous crop only helps in establishing the existing types and cannot bring any appreciable change in the material.

N. Hjalmar Nilsson (1901–1909, see Akerman *et al.*, 1948) and his co-workers at Svalof realised the importance of individual plant progenies for fixing the selected types and established the well known "plant progeny test" which is the main basis of modern pedigree selection in the self-fertilised crops. Subsequently in 1903, Johansen's brilliant experiment with beans explained the genetic basis of pure lines and the limitation of selection within a line.

The Pure Line Theory

Johansen (1903), a Danish biologist, defined "pure lines" as the progeny of a single self-fertilised homozygous individual. Johansen studied the variation in beans for seed weight in a mixed lot and in 19 lines established by growing the progenies of seeds representing a wide range of seed weights. The details of his experiments are given in most textbooks, therefore, only salient features of his studies and the conclusions drawn from them are mentioned here. He observed:

1. Seeds from the progeny of a large seed were larger than the seeds from a progeny of a small seed. The commercial seed lot was a mixture of purelines and selection was effective in separating the lines homozygous for different genes controlling the seed size ranging from small to large seed.
2. Further selection for seed size within a pure line was not effective.The mean weight of each line remained remarkably constant from generation to generation in spite of the observed variation in the seeds within a line.
3. Observed variation in the seed size of the original population, and of the isolated lines, represents heritable and non-heritable variation (environmental). In the original seed stock, variation was both hereditary and environmental. The variation within a pure line was due to the environment only and, therefore, selection within a pure line was not effective in separating the seed lots of different sizes in subsequent generations.

These observations clearly showed that the success of breeding improved varieties by selection in self-pollinated crops depends on the efficiency with which the heritable and non-heritable components of variation could be recognised and heritable variation is selected for.

Modern Breeding Methods

Breeding methods and approaches depend on the objectives (short and long term), stage of a breeding programme, available breeding populations or germplasm, genetic determination and inheritance pattern of the characteristics to be improved and breeder's resources. As indicated by the foregoing discussion, early plant breeders exploited natural genotypic variation in crop plants to their advantage by introduction, acclimatisation and selection (mass or single plants).

However, when natural genotypic variability for the desired characteristics becomes limiting, a breeder has to create the needed genetic variability, which can be selected upon and fixed into agronomically desirable genotypes. Rediscovery of the Mendel's laws in 1900 and subsequent studies in the fields of genetics and cytogenetics accumulated necessary information on segregation, gene interaction, linkage and recombination, which laid the basis for creating new genotypic variability by hybridisation on one hand and by induction of mutations, chromosomal alterations and ploidy manipulations on the other. Concurrently, developments in statistical methods and quantitative genetics have provided necessary techniques for improving the precision of recognising heritable quantitative variation for characters such as yield and quality. The application of genetic and cytogenetic techniques to create new variability and statistical and biometrical approaches together constitute the modern plant breeding methods.

The different phases of plant breeding approaches in a breeding programme are shown in Fig. 2 and the methods and procedures are discussed in detail in the following.

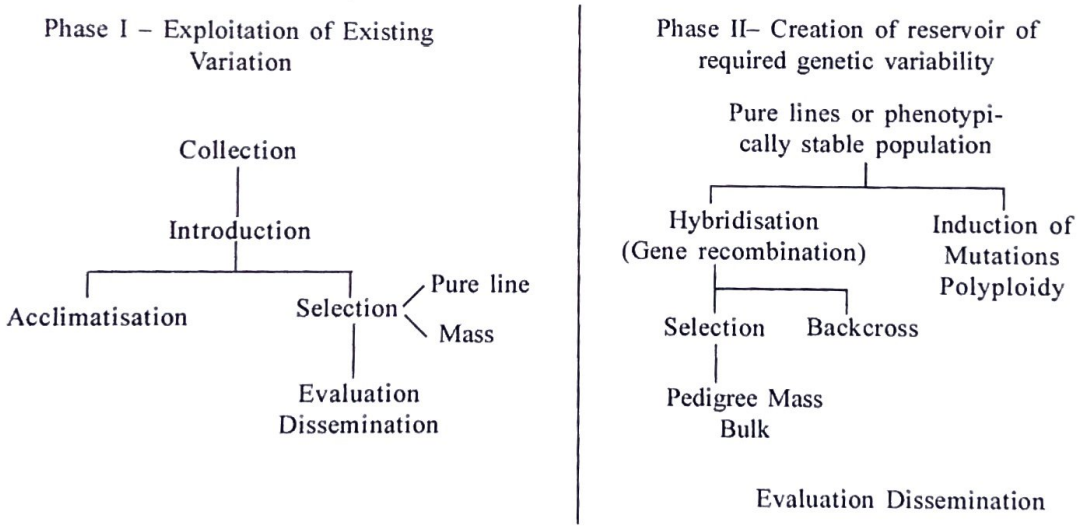


Fig. 2. Phases of plant breeding programmes

Introduction

Occasional movement of early farmers and communities introduced the crops and varieties used by them into the new area of their migration. Such introductions, and chance hybridisation in nature between them and the existing types provided the needed reservoir of genetic variation for selection (adaptation) and development of new varieties with higher yielding ability. Subsequently, traders, invading armies and colonisers introduced new plants from one region to another. The discovery of the Americas resulted in the introduction of maize, beans,

squash, groundnut, potatoes, tomatoes, chillies, tobacco and American cotton into Europe and Asia. Introduction of plantation crops such as sugarcane, bananas, and coffee in the Americas and cotton, groundnut, tobacco and rubber in Asia and Africa provided the needed raw materials for the Industrial Revolution in Europe.

In the forties (1946) introduction of Norin-10, a Japanese dwarf variety of wheat, into the U.S.A. by USDA Agronomist S.C. Salomon (Reitz and Salomon, 1968) resulted in the development of record-breaking high-yielding winter wheat varieties *Gaines* and *Nugaines*. Subsequently, in the mid-sixties large-scale introduction of high-yielding dwarf varieties of wheat [*Lerma Rojo*, S308 (*Sonalika*), *Sonora* 64] and rice (TN-1, IR-8, IR-20, IR-24 and IR-36) based on the dwarfing genes of *Norin*-10 and *De-geo-wu-gen* respectively has been the basis of the green revolution in India and Pakistan. In India, besides wheat and rice introductions, sunflower varieties from Russia and soybean from the U.S.A. have opened new production areas in rainfed agriculture of central and peninsular India and have provided an industrial base in the region.

Historically, plant introduction meant transport of collected seeds or plants from one production area to another. In the initial stages of agriculture, production areas were restricted niches of crop species. Any movement of plant material from one settlement to another was a new introduction. Now when agriculture has expanded so much, it is difficult to delimit boundaries of production area of a crop plant or its varieties within a country or even countries in the same continent. Ideally, transport of seeds or plants from one ecological zone to another and their establishment in a new environment is plant introduction. However, the widely accepted definition of plant introduction is a crop species or a variety or a genetic strain (carrying a gene or genes for characters not easily available in the area) introduced from one country to another. This definition is based on the distinction that movement of seeds within a country is easy, while movement of seeds and plant materials between countries is regulated by quarantine regulations and procedures.

As shown in Fig. 2 introduction and selection mark the beginning of any plant breeding programme, and contribute a great deal towards the development of varieties with superior performance. In a highly advanced breeding programme introductions are generally used as donors of gene or gene combinations required for further improving an existing well adapted genotype lacking in one or more characteristics, such as susceptibility to a disease or quality trait.

Since development of uniform high-yielding varieties is replacing the land races in the farmers fields and the centres of diversity are shrinking due to increased agricultural activity, we may soon find that the genetic variation in the available

crop plants is not adequate for sustained improvement to meet the challenges posed by dynamic disease and pest complexes and the changing crop management practices. To avoid such a situation, activity of germplasm collection, evaluation and maintenance is receiving attention at the international level, so that the needed genetic variability is captured and stored for introduction when required in the future breeding programmes. The International Board for Plant Genetic Resources (IBPGR), with headquarters in Rome has established the following regional programmes for germ plasm collection and maintenance:

1) South-east Asian Programme.
2) South-west Asian Programme.
3) European network under the auspices of Eucarpia.
4) National programmes of the U.S.A., the erstwhile, Brazil and India.
5) Genetic resources programmes of the international (CGIAR) institutes.
   a) CIMMYT — International Maize and Wheat Improvement Centre—Mexico.
   b) CIP — International Potato Centre—Peru.
   c) IRRI — International Rice Research Institute—Philippines.
   d) IITA — International Institute of Tropical Agriculture — Nigeria.
   e) ICRISAT — International Crops Research Institute for the Semi-Arid Tropics (deals with sorghum, pearl millet, groundnut, chick-pea and pigeon pea) — India

In India, the National Bureau of Plant Genetic Resources, with its head-quarter at Delhi, has the responsibility for plant exploration and germplasm collection. The bureau has set up 10 regional centres in different agro-ecological regions for collection and maintenance of the germplasm of different crop plants. Details of handling and evaluation of the germplasm introductions are given in the chapter on germplasm resources.

Acclimatisation

It has been observed that when first introduced, certain populations of crop plants appear to be poorly adapted to the new climate but after a few seasons do well and become more productive. This ability of a population to recover and adapt, to a new climate situation is referred to as acclimatisation. It was first thought that plants slowly acquire an ability to withstand the new climatic conditions during exposure of a few seasons. However, an understanding of the genetic heterogeneity of the populations indicated that the process of acclimatisation is a population phenomenon and not an acquired characteristic of individual plants to withstand adverse climatic conditions in a new area. As a

heterogeneous mixed population is exposed to new climatic conditions (temperature, moisture, disease etc.) genotypes not suited have a selective disadvantage and the types capable of coping with the new environmental conditions increase in frequency generation after generation due to better fitness. Once all the unadapted types are eliminated the mean performance of the population is better than it was in the first few years. Therefore, the process of acclimatisation is the result of genetic shift towards the adapted types out of a variable population subjected to environmental stress. The efficiency of acclimatisation process depends on: (a) the extent of genetic heterogeneity in the population; (b) the mode of reproduction (pollination) of the species; (c) duration of the life cycle of the species; and (d) the nature and intensity of environmental stress.

In a cross-pollinated crop, acclimatisation will be more rapid than in a self-pollinated crop, because of the greater frequency of the recombination, assuming that the recombination will lead to productive genotypes with more favourable adaptation. On the other hand, a pure line, but for mutation and limited outcrossing, will change only slightly. In a short duration crop, there are more chances of recombination because several generations can be grown in a given span of time and, therefore, more opportunity is available to increase the frequency of favourable adapted types.

The nature and extent of environmental stress will determine the extent and speed with which acclimatisation will establish a population in a new environment. If a newly introduced population carries genetic heterogeneity for susceptible and resistant genotypes to a particular disease, severity of the disease will determine how quickly all susceptible types are eliminated and the established population consists only of resistant types. As such, acclimatisation is not a breeding method. Instead, it explains the process by which new introductions gradually become better adapted and established as superior performing populations.

Selection

Selection is the process by which individual or groups of individuals are sorted out from a heterogeneous mixed population. Such a population may be an adapted land race or a segregating generation of a hybrid, Breeders of self-pollinated crops practice: (1) mass selection, and (2) single plant or pure line selection.

Mass selection

In ordinary terms, mass selection refers to the age-old practice of harvesting desirable heads or plants and compositing the harvested seed to produce the next generation.

Poehlman (1979) emphasised that mass selection is based on phenotypic selection without progeny testing and indicated the following drawbacks of the method:

1) It is not possible to know whether the plants being grouped are homozygous or heterozygous. Since the heterozygous plants will segregate in thefollowing generation, phenotypic selection may need to be repeated.
2) With mass selection it is not possible to know whether the selected phenotype is superior in appearance due to hereditary characters or due toenvironment.

However, the recent practice of selecting individual plant progenies and compositing the seed of similar looking progenies helps in improving the efficiency of mass selection to some extent. According to Allard (1960) the main difference between pure line selection and mass selection in self-pollinated crops relates to the number of lines retained. With pure line selection the derived type consists of a single line. With mass selection the majority of the lines selected are retained and composited in one population consisting of a mixture of pure lines. The derived population attains practical uniformity for easily observable characteristics of agronomic importance, such as, grain colour, plant height, seed size, maturity period and resistance to diseases.

With the advent of pure line theory in 1903, breeders of self-pollinated crops laid emphasis on the development of pure line varieties and mass selection has been mainly used to purify a mixed variety or a seed lot in seed production programmes. The steps of varietal purification by mass selection are as under:

1) Select 200 to 2,000 individual plants representing the main varietal features. The number of plants will depend on the availability of resources. Small sample may adversely affect the adaptability of the variety.
2) Grow individual plant progenies in three or four row plots and observe them for uniformity of various phenotypic characters.
3) Bulk the similar looking uniform progenies for seed increase.

If a large number of progenies have been retained and only a few have been discarded then the bulk can be used for multiplication of the variety without further testing and evaluation for yield and adaptation. However, if the number of progenies discarded is high (over 25 per cent) then it will be desirable to evaluate the composited seed lot for adaptation and yielding ability to ensure that the new lot is not inferior to the original variety under purification.

In recent years, a better understanding of quantitative genetic variation and the concept of heritability and gene action have led to a renewed interest in mass selection as a method of breeding improved varieties of self-pollinated crops. Frey (1967), Romero and Frey (1966) and Hartwig and Collins (1962) have successfully used the mass selection procedure for improving plant height, seed

size and protein content, respectively. According to Frey (1976), mass selection is a very effective and inexpensive technique for increasing the proportion of desired genotypes in bulk populations of self-fertilising plants.

The success of the method depends upon high heritability, that is the presence of additive gene action and minimal influence of genotype × environment interaction on the expression of the selected trait. Gardner (1961) suggested phenotypic stratified mass selection for improving yield of maize population. He space-planted 8,000 to 10,000 plants in a uniform irrigated field of less than 0.5 ha and subdivided the field into blocks (grids), each with more or less uniform environment. Each grid had 40 plants and he saved the top 10 per cent for yield from each block. He observed an advance of 2.7 per cent per cycle for 11 cycles.

Matzinger and Warnsman (1968) working with tobacco, an autogamous plant, suggested that the best environmental control would be achieved by practicing within row selection. They selected three plants from each row of 20 plants each. The selected plants were intermated in random pairs to provide seed for the next cycle. They obtained a linear increase of 4.29 per cent per cycle for four cycles.

There are not many reports on the efficiency of mass selection for improving yield. However, with reasonable control on the environmental variation as indicated above, the method can be very effectively used for increasing the frequency of genotypes for certain highly heritable characters in the early generations of a segregating population and may help indirectly in increasing the yield of the population. Subsequently, selection for yield can be exercised on the basis of progeny testing and yield tests. Frey (1967) reported that simple mechanical screening for wide and heavier seeds in oats increased the grain yield by 9 per cent in four cycles. Seed weight within a certain range has positive association with yield in many cereal crops and, therefore, can be a very convenient and useful character for mass selection in the preliminary stages of selection in a segregating population.

Tiyawalee and Frey (1970), Chandhanamutta and Frey (1973) and Matzinger *et al.* (1976) have cautioned against antagonistic correlations due to which selection for one desired trait may result in undesirable changes in another. For example, selection for wide seeds and heavy panicles in oats resulted in increased yield but plants in the selected population became taller and late (Chandhanamutta and Frey, 1973). In tobacco, selection for short plant height reduced leaf number. Conversely, selection for increased leaf number increased plant height (Matzinger *et al.*, 1976). However, information on character associations has accumulated in most crops and, therefore, where characters are antagonistically

associated, index selection as suggested by Matzinger *et al.* (1976) that is simultaneous selection for both the characters, short height and more number of leaves in tobacco may be helpful.

In cereal crops such as wheat, rice and oats, where late and tall plants have competitive advantage over short strawed and early types, which have high yield potential under good management, a two step selection procedure will be useful for avoiding undesirable antagonistic correlated responses. In the first step, remove undesirable tall and late plants from the population (use large population of 5,000 to 10,000 plants). In the second step, select for desirable seed size mechanically and then evaluate individual plant progenies for yielding ability before compositing the seeds of phenotypically similar promising plant progenies.

The combination of simple mass selection with progeny compositing is easy in handling and cheaper in cost. A variety developed by this procedure of mass selection will be phenotypically uniform for agronomic purposes and identification, but will carry a considerable degree of genetic variability for quantitative characters. Such varieties have the necessary flexibility for adaptation and improvement over years. In other words, mass selection conserves considerable genetic variability in the population, which is useful for slow and gradual improvement over time.

Pure line varieties

Individual plant selection for the purpose of developing a pure line variety is practiced in two situations.

1) Development of pure lines from a well-adapted land race or a wellestablished mass selected population.
2) Development of a pure line from a segregating population of a hybrid. The procedural details of individual plant progeny selection in the two situations are influenced by the fact that:
    a) In case of land races of well-established mass selected populations,most individual plants, barring a few chance heterozygotes are homozygous at majority of loci.
    b) In case of segregating populations, the individual plants are heterozygous in the beginning and attain homozygosity in successive generations as discussed earlier.

In case of land races, selection of individual plants and growing their progenies quickly establishes a pure line due to the high level of homozygosity of land races. In case of segregating populations individual plant selections have to be carried for at least five to six generations of selection to achieve the desired

level of homozygosity of a pure line. Also, the number of individual plant progenies to be evaluated in case of a segregating hybrid population is in the order of 3,000 to 10,000 plants so that the maximum number of desired genes are recovered in homozygous condition. Long adaptation in case of land races narrows the genetic variability for quantitative characters responsible for fitness and productivity, and therefore it is easy to recover desirable pure lines with 200 to 500 plant progenies. The detailed selection procedure and evaluation of individual plant progenies in case of hybrid segregating population will be discussed under the subtopic of hybridisation.

The selection of individual plants and development of pure lines from land races is based on the fact that the mean performance of genotypes in a population is always lower than the performance of the best genotype present in it. Therefore, selection is aimed at identifying the genotypes, which give the best performance in the population. The steps followed to achieve this are:

1. Select 100 to 500 single plants depending on the availability of the space and resources.
2. Grow single plant progenies in three or four row plots with 30 to 50 seeds per row depending on the availability of seed. Select 20 to 100 promising uniform looking progenies and in each selected progeny select the five best plants.
3. Grow individual plant progenies for the selected families from the previous year in a similar manner under normal field conditions.

   Also a row of each of the individual plant progenies is grown in the disease nursery, if a particular disease is important in the area. First select the best families on the basis of uniformity, disease reaction, and other desirable characters; and then select the most promising one or two progenies in each of the selected family. Select three to five outstanding plants in each of the progeny for growing the individual plant progenies next year.
4. Repeat step 3 for another year and finally select 25 to 50 single plant progenies. Select five to 10 plants in each of the selected progenies separately for seed increase and bulk harvest for replicated yield tests.
5. Replicated yield tests should be conducted at three to five locations and may be repeated for two to three years to identify the best pure line for its performance in the general area of adaptation of the variety.

In recent years the procedure has been successfully used in developing superior pure lines of pigeon peas from varietal populations of UPAS "120 and T" 21 in the pigeon pea breeding programme at ICRISAT (Gupta *et al.*, 1980).

Most breeding programmes for selfpollinated crops emphasise the development of pure line varieties because:

1. A pure line variety is highly uniform in appearance and quality of the product.
2. A pure line adapted to specific agro-ecological and management conditions is likely to give better performance than a mixed population of genotypes.
3. A pure line variety is easy to identify and maintain.

However in recent years evidence has accumulated, which shows that extreme uniformity of a pure line is unnecessary for a variety. Highly uniform pure line varieties when grown on extensive areas, easily succumb to races of disease pathogens.

Hybridisation

The term hybridisation refers to crossing of two genetically different individuals. As a breeding procedure, it combines the characteristics of two varieties and provides an opportunity to select plants with desirable features of both the parents through recombination in the segregating progenies.

The idea of hybridisation originated as early as 1694, when Camerarious, for the first time, demonstrated sex in plants and suggested crossing to get new types (Smith, 1966). Historically, Kolreuter (1760"1766), Thomas Andrew Knight, (born 1759), John Goss (1820), Gartner (1849) and Naudin (1865) studied plant hybrids during the eighteenth and nineteenth century (Hayes *et al.*, 1955). However, hybridisation as a planned plant breeding approach came into practice only after the rediscovery of the Mendel's laws in 1900, when genetic consequences of hybridisation were understood in terms of segregation and recombination of genes governing individual characteristics.

Biffen reported in 1905 that resistance to strip rust of wheat was controlled by a single recessive gene and it was possible to develop a resistant variety by crossing the resistant and the susceptible parents. This marked the beginning of extensive use of hybridisation for correcting major highly heritable defects, such as susceptibility to a particular disease, seed colour, flowering duration and plant height, of crop varieties. Subsequently, with the under-standing of inheritance of quantitative characters, the influence of environmental effects and application of statistical techniques, procedures were developed to recognise lines with superior yield and quality from out of segregating populations of the hybrids. The success of a hybridisation programme to obtain improved varieties depends on: (a) clear definition of breeding objectives, (b) choice of parents and mating system, (c) effective handling of segregating population and selection of desirable types, and (d) proper testing and evaluation of promising selections.

Objectives

All crossing programmes aim at combining the desirable characters of two or more varieties, germplasm lines or species. Since no variety is perfect, segregating populations from a cross give an array of recombinants, which may combine the good and bad features of the two parents. Occasionally, there may be transgressive segregation leading to a genotype superior to both the parents. A breeder is interested only in performance better than the existing variety and does not wish to lose on other aspects while improving a specific trait. Objectives of a varietal development programme depend on the need for a new variety to cope with the complex requirements determined by agro-ecological, biotic (diseases and pests), crop management and quality considerations of the product. It is impossible to meet all the requirements in a new crop variety simultaneously. Therefore, it is essential to sub-divide the overall objective of developing a superior all round good performing variety into simple, well defined, specific objectives in terms of priority. For example, the remarkable success of high yielding rice and wheat varieties, initially developed at IRR1 and CIMMYT, is credited to the fact that breeding objectives were simple and clearly defined. In the case of rice the main objective was to develop a dwarf non lodging variety with high yield potential under high level of management. The programme quickly developed the variety IR8 with a record yield potential. The consideration of disease resistance and grain type etc. were attended to subsequently. Similarly, in case of wheat the first priority was given to dwarf, nonlodging high yielding types; resistance to rust and emphasis on other attributes such as grain colour, and quality were deferred for a later stage.

Identification of a simple objective in varietal improvement helps in identifying appropriate parents and effective selection of desirable recombinants. For clear identification of objectives and determination of priority, a breeder should have good knowledge of the general goals of breeding a particular crop, of the problems of growing the crop, of the performance of the existing commercial varieties and their weaknesses, of available breeding lines and germplasm, and of the inheritance of the characters to be improved.

The objectives can be classified into short- and long-term ones. A short-term objective may be to remove a particular defect, such as disease susceptibility of an otherwise very good variety. This can be achieved by hybridising the two parents with minimum difference in other desirable characters except the difference in disease reaction. A long-term objective may be making an overall improvement in the performance level and other attributes. For this, one may have to resort to wide crosses or different types of multiple crosses.

Since development of a commercial variety takes anywhere from 10 to 15 years and making crosses is only the beginning of the long process, proper planning at this stage is essential for the success.

Choice of parents and mating systems

Choice of parents for a hybridisation programme is critical for its success and requires careful and critical evaluation of potential parents for various attributes such as yielding ability, disease resistance, adaptation, quality of the produce, and morphological features relevant to crop management practices. Generally a breeding programme has to choose parents for: (a) crosses for developing a variety for use in the near future, and (b) crosses for enhancing or developing germplasm lines for effective use in a breeding programme in the long run. Often exotic germplasm, when introduced in a new environment, is not well adapted. Therefore, it is desirable to incorporate useful genes from such exotic germplasm into agronomically adapted and desirable local types. The derived lines can then be used in crosses for varietal development.

For meeting immediate varietal needs, crosses are made between two adapted varieties or advance breeding lines. In an on-going programme usually data on yield performance, quality characteristics, special attributes of disease or pest resistance and breeders general knowledge of the existing varieties of the crop help in the choice of parents. However, for a new breeding programme it is essential that the breeder initiating the programme should thoroughly evaluate the existing varieties and possible germplasm introductions for their yield performance, adaptation and other attributes such as quality and disease and pest resistance.

Since new strains are intended to have superior yield potential than the existing varieties, one of the parents is invariably the adapted variety of the area. The other parent is primarily chosen for complementing the specific weakness of the variety, which needs to be replaced. The general adaptation and performance of the donor line, as far as possible, should also receive attention. Selection of parents for simply inherited characters controlled by a few major genes, such as disease resistance or plant height genes in wheat and rice, is easy and does not require any special technique. However, selection of parents for improving quantitative characters, such as yield and crop quality which have highly complex inheritance controlled by many genes (polygenes) with small effects and high genotype × environment inter-action, has been rather difficult.

In the fifties, analysis of diallel crosses described by Hayman (1954), Jinks (1954; 1956) and Griffing (1956) attracted the attention of several workers and a large number of studies in different crops have been conducted to determine

the value of diallel cross analysis to predict the performance of parents and to identify promising crosses. Gilbert (1958) made a critical appraisal of the value of diallel crosses in plant breeding and concluded that diallel cross gives information that cannot be obtained from the parents as such, but this additional information is not equal in importance to the parental yields. To paraphrase a famous quotation, "like begets like, but some are more like the others". The performance of the parental varieties themselves gives valuable prediction of the relative behaviour of the crosses, but the diallel cross does give further information.

Lupton (1961) conducted diallel cross analysis involving six varieties of wheat and observed that little advantage was gained from the diallel analysis, since the promising crosses predicted and confirmed by observations in F_3 and F_4 generations could have been conveniently selected on the basis of yield figures of the parents alone.

Since the genetic variance in self-pollinated crops is predominantly additive (Matzinger 1963, Moll and Stuber 1974 and Hallauer 1982) the general combining ability of a parent is likely to be reflected adequately in the parental performance of the trait. Diallel studies in most self-pollinated crops have shown good agreement with *per se* performance of the varieties and their general combining abilities. Busch *et al.* (1974) in wheat and Hamblin and Evans (1976) in dry beans *(Phaseolus vulgaris* L.) have concluded that the performance of parents is a good indication of the potential of a cross.

In the light of the above discussion it may be concluded that the best approach to select the parents will be to conduct a varietal trial of potential parental lines. In the beginning of a breeding programme the number of lines to be evaluated may be sufficiently large and the number entered in the test will depend on the resources of the breeding programme. If the number is large (around 100), the lines could be tested in two to three replications at three to five locations. If a multilocation test is not possible then testing will have to be continued for two to three years at one location. Eventually one may settle for 15 to 20 top lines. These selected lines could then be crossed in a fractional diallel as suggested by Gilbert (1958) and Lupton (1961) or in a line × tester top-cross system. Four to six best adapted varieties of the area should be used as the tester parents, since the varieties intended to be developed should have superiority over the existing ones. In self-pollinated crops it may not be possible to obtain sufficient top-cross F_1 seeds for a replicated test and it would be desirable to advance the top-crosses to F_2 generation to conduct the test with appropriate field design.

Besides selecting the parents on their yield performance and general and specific combining abilities in the partial diallel crosses or line × tester crosses, it is

desirable to analyse the potential parents for principal yield components such as tiller number or number of branches, grains per panicle or pod number, and grain size. Woodworth (1931) suggested that yield might be increased in small grains by selecting for the components of yield and that parental varieties should be selected on the basis of their component attributes. Grafius (1956; 1964) argued that it would be easier to select for the individual yield component than yield itself and maximum gain in yield can be obtained through the increase in the components which is the smallest. For example, in cereal crops increase in grain weight is likely to result in more gain in yield than an increase in number of spikes per unit area or number of grains per spike.

However, it has been argued that increase in an individual component may not necessarily result in higher yield because of physiological balance between source and sink resulting in component compensation. Adams (1967) has shown that component compensation and negative correlations arise in response to competition between developmentally flexible components. In the absence of stress, the correlations are trivial. This indicates that the components are genetically independent to some extent. It is true that component compensation will not allow unabated increase in yield by improvement of individual components, but the compensation is not necessarily complete. Component complementation of parents allows an opportunity to have a reasonable compromise and balance between one or two components result in high yield. The optimal genetic level for each component would differ depending on the type of the environment encountered (Grafius, 1965).

Rasmusson and Cannell (1970), working with barley, observed that selection for yield through components was very effective in certain situations. Genetic as well as environmental factors were responsible for the observed phenotypic correlations between the components of yield. They postulated that the optimum genotypic level for kernels per head and the number of heads will vary depending on environment, but the optimum for kernel weight will be near its genetic maximum in most situations. Selection for one component did not affect the other components necessarily. Knott and Talukedar (1971) have shown that improvement in seed weight of the well-known variety Thatcher resulted in an increase of 13.3 per cent in yield. Similarly, Grafius *et al.* (1976) reported that the major part of the difference in mean yield between crosses could be attributed to component complementation between the two parents.

Bhadouria *et al.* (1977) studied combining ability of wheat-agropyron translocation derivatives and observed that the existing popular varieties of wheat were good general combiners for grain yield and 1000 grain weight while translocation lines were good for tillers per plant. Specific crosses having the

highest yield were between the standard varieties and the translocation lines, because of the desirable complementation of the important yield components.

In the light of the above discussion one may conclude that the choice of parents should first be based on their *per se* yield performance and then on critical evaluation of the components of yield so that while choosing cross combinations due consideration is given to complementation of components from the two parents.

It should also be remembered that choice of parents based on their yielding ability alone is likely to result in a very narrow genetic base, since co-adapted gene blocks or linkats, as described by Demarly (1979), responsible for high yield of the two parents are not likely to be different. Consequently it is not possible to obtain higher yielding transgressive segregants from such crosses. Saffeeulla (1977) has discussed in detail the effects of extensive use of derivatives of the dwarf Mexican wheat cross 8156 and rice varieties IR8 and Taichung Native"1 in India in relation to vulnerability to diseases and pests. The importance of genetic diversity for improving yield potential *per se* has been emphasised by several authors and reviewed by Frey (1971).

Mating system

Hybridisation programmes in self-pollinated crops mainly involve bi-parental crosses. Such crosses are attempted with the objective of removing a particular defect in an otherwise superior well adapted variety and are of two types: (1) adapted × adapted high yielding variety crosses, and (2) adapted x unadapted germplasm line crosses. Crosses of the first category resulted in sister lines or varieties, which had a narrow genetic base, since in a given area high yielding ability was restricted to a few lines and these were repeatedly used as parents for developing new varieties. The second category crosses often resulted in intermediate types with inferior yield than the adapted high yielding variety, unless the character added from the unadapted parent was a major impediment; such as resistance to a particular disease in an endemic area.

Harlan and Pope (1922) suggested the backcross method to conserve the yielding ability of a well adapted variety, while incorporating resistance from an unadapted parent. Briggs (1930) started an extensive backcrossing programme to develop bunt resistant varieties of wheat. Backcross derived lines apparently looked similar to the agronomically superior recurrent parent and had the advantage of resistance under disease pressure.

Biparental crosses have served the purpose of combining simply inherited characters from two parents and have been useful in step by step progress by combining several characters such as earliness, disease resistance, grain quality,

and yielding ability. It involved careful selection and fixing of the best segregates and yield testing them for some years before starting a new cycle of recombination. The whole process is time-consuming. Some of the winter wheat varieties developed by the Swedish Seed Association by this approach took 50 to 60 years (Akerman and Mackey, 1948).

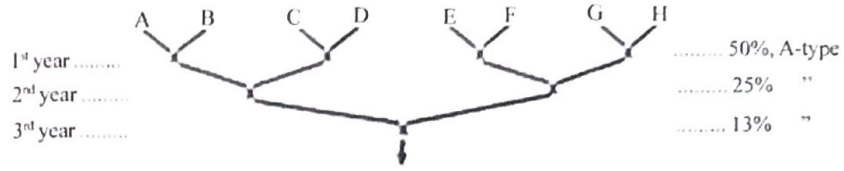


Fig. 3. Convergent cross according to the principle of maximum recombination

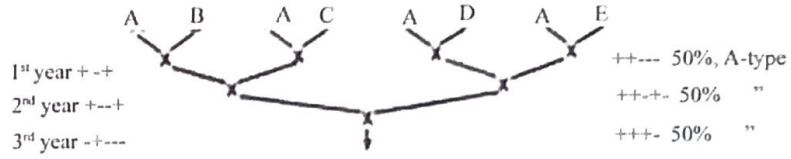


Fig. 4-A. Convergent cross according to the principle of transgressive recombination

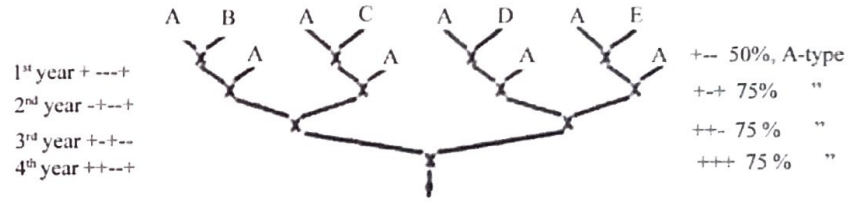


Fig. 4-B. Convergent cross according to the combined principle of transgressive recombination and incomplete backcross.

Harlan *et al.* (1940) proposed a scheme of multiple convergent crosses involving eight to 16 parents to overcome the restrictive recombination obtained in a biparental cross and to expedite the process by intercrossing the Fls (Figs. 3 and 4). Theoretically, the objective of multiple crosses is to obtain recombination of genes from several parental strains. However, Akerman (1946) argued that, in practice, it will be impossible to combine a large number of genes from many parents, since a cross involving eight parents (Fig. 3) with one valuable gene each which does not exist in other seven varieties will require 1,31,000 Fl seeds in the last cross to ensure a 50: 50 chance to have all the eight genes together in one genotype.

Another limitation of a multiple cross involving many parents, pointed out by Mackey (1954), is that the breeder will be forced to include a number of unadapted strains, because it is not easy to find many well adapted parents having different genes. Inclusion of unadapted parents in a crossing programme

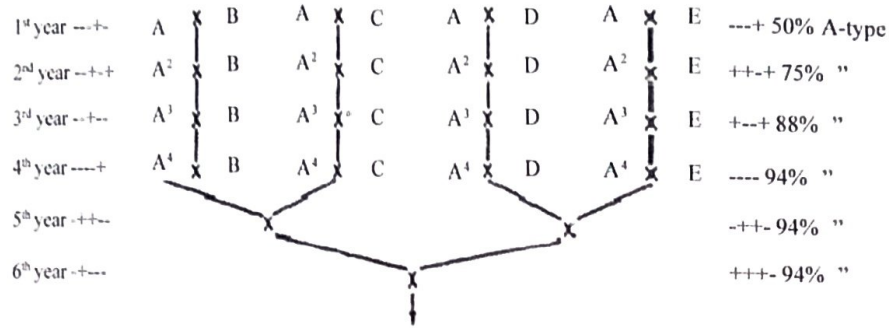


Fig. 4-C. Convergent cross according to the combined principle of transgressive recombination and backcross (convergent backcross).

is likely to disrupt the genetic background of good varieties that took several years, or even decades, to assemble. He adopted a modified version of convergent crosses called "convergent crosses according to the principle of transgressive recombination as shown in Fig. 4A". In this approach a very good variety is chosen as persistent crossing partner at every stage of the cross procedure, which results in 50 per cent of the genes from the persistent good variety. This procedure was further modified by Mackey by including different degrees of backcrossing in the programme as shown in Fig. 4B and C to ensure 75 per cent to 94 per cent of the genes from the adapted good variety. The procedure is known as convergent backcross.

The mating systems adopted in breeding programmes have two objectives:

1) To identify promising parents and potential cross combinations based on general and specific combining abilities.

As discussed earlier diallel crosses and line × tester top-cross matings are used for choosing the probable parents and cross combinations to be pursued for selecting improved lines in the segregating generations.

2) To combine a number of desirable genes from more than two parents. The mating schemes to achieve the second objective are:

A) Sister line crosses

Intercrossing of selected superior lines from a cross serves the same purpose as recurrent selection in cross pollinated crops that is, the accumulation of a large number of genes with additive effect in a population by successive generations of recurrent selection. In this procedure high yielding lines isolated from a cross are intercrossed to give sister line biparental crosses. The approach is based on the reasonable expectation that the sister lines will all contain different plus genes. The most obvious disadvantage of the method is that each cross is carried till homozygosity is established and then the next cycle is initiated. The

process, therefore is very slow. Each cycle may take six to ten years. Two or three cycles of such selection and crossing should assemble the major linkage groups determining maximum expression of a character in one or a few homozygous lines. Since the procedure is very slow and time consuming it is not desirable that most crosses be handled in this manner. Once a valuable cross has been identified it would be desirable to utilise its full potential by exploiting sister line crosses for producing transgressive segregants and lines of high yield potential (Palmer, 1953).

B) Convergent crosses of different types

These have been discussed earlier.

C) Complex crosses of single backeross type or three-way crosses and double crosses

As discussed earlier Mackey suggested varying degrees of backcrossing with the adapted parent in order to incorporate desirable genes from exotic germplasm. The degree of backcrossing will depend on the extent of diversity of the two parents. If the exotic parent is a weedy wild form then several backcrosses may be required to obtain agronomically desirable lines. Lawrence and Frey (1975) working with crosses between *Avena sativa* and *Avena sterilis*,observed that recovery of good *A. sativa* lines with improved yield from *A. sterilis* sources was obtained in BC2 to BC4 generations of back-crosses with the *A. sativa* parent. Ertl and Fehr (1985) reported that three backcrosses to the cultivated parent were necessary to obtain a reasonable number of lines similar to the recurrent parent in crosses between *Glycine max* X *Glycine soja*. They concluded that introgression of *G. soja* germplasm into the two soybean cultivars was not an effective method for increasing their yield potential. Since backcrosses to the adapted parent limit the range of parents and are not likely to improve the performance over the recurrent parent, three-way crosses have been tried. The third parent is another adapted high yielding type. This parent serves the purpose of a backcross in increasing the proportion of adapted germplasm but widens the scope of combining desirable genes from another good cultivar. Thorne and Fehr (1970) have observed that the populations of three-way crosses of soybean had larger genetic variance than for two-way cross populations and the three-way crosses produced more superior lines for yield than the two-way crosses.

Rawlings and Cockerham (1962) suggested that a variety having the best general combining ability or average effect (high *per se* performance and adaptation) should be used as the third parent for a three-way cross. Such crosses have an element of gamete selection, since the superior performance of TC F_1 plant or

TCF_1 (Triple cross Fi) family reflects the combination of a superior gamete from the segregating single cross with the genotype of the nonsegregating variety used as the third parent. As argued by Stadler (1944), the frequency of superior gametes is much higher (q^2) than the frequency of superior zygotes (q). Therefore, three-way crosses provide a more efficient mating system for obtaining lines with high performance. However, in such crosses [(A×B)×C], the choice of the third parent (C) will be of crucial importance, since C will constitute 50 per cent of the germplasm, while contribution of A and B will be only 25 per cent each.

Besides three-way crosses, double crosses involving four different parents [(A × B) × (C × D)] are also used to extend the range of parents and achieve recombination of desirable genes from different diverse parents. The use of three-way or double crosses is limited to a great deal by the requirement of a large number of crossed F_1 seed. The seeds of a three-way cross are the products of the gametes of a single cross and that of a variety. In a double cross, gametes of two single crosses are involved. These gametes are genetically different from each other. The resulting F_1 generation itself constitutes a segregating population similar to the F_2 of a single cross. Therefore, the number of crossed seeds sets the limit to possible recombination of genes available in the population. For a double cross more than 1,000 crossed seeds will be required to expect one plant with desirable alleles at five loci contributed by the four parents. Sneep (1977) concluded that use of double crosses (multiple crosses) for combining many factors in a genotype is practically not possible.

D) Diallel selective mating

Jensen (1970) proposed a selective mating scheme to attain recombination of desirable genes from multiple parents (Fig. 5). It involves: (a) crossing of a select group of F_1s in a diallel set or a partial diallel set, (b) crossing of the selected F_1s in a diallel or partial diallel, and (c) selection in population of F_1 crosses. The F_1 cross populations are advanced to F_2 and some form of mass selection is carried out and many random crosses are made among the selected plants. Selection and intercrossing may be continued for several generations to maximise heterozygosity and recombination. This system provides for (a) broadening the genetic base of the population through use of multiple parents, (b) an opportunity of recombination of genes and breaking of linkage through intercrossing of selected types at different stages, (c) an opportunity to introduce the new germplasm at any stage of the programme, and (d) opportunities of fulfilling short term and long term objectives by following conventional selection approach at any stage in the programme.

The system has not been critically evaluated for its success and efficiency. The apparent drawbacks of the system are (i) the requirement of a large number of crossed seeds from the diallel and partial diallel crosses of the F_1s, and (ii) absence of effective selection of individual plants for further intermating, particularly for characters with low heritability. There is a need to develop operational details of handling the system in different crops and determining its efficiency for improving quantitative characters.

Handling of Segregating Population and Selection of Desirable Genes

In case of a self-pollinated crops the objective is normally to establish a pure line, for release as a commercial variety. Hybridisation between the chosen parents is only the beginning of a long process of selection and evaluation of recombinant genotypes. Breeders use a number of variations and modifications in the procedures of handling segregating generations depending on the availability of resources, breeders own experience, objectives of varietal improvement and the mating system used. The success of a breeder depends on his ability to take short cuts to attain the objectives and economise on resources. It is difficult to spell out all the detailed minor variations used by different plant breeders. However, the main principles and approaches are discussed. An association with a successful breeder helps in picking up operational details.

In handling of crosses selection is exercised at two levels (1) selection between crosses and (2) selection within the segregating population of a cross.

Selection between crosses: Early elimination of poor crosses helps in efficient utilisation of resources and allows handling of a reasonably large segregating population of a promising cross required for obtaining the desirable recombinants for quantitative characters.

Popularly, plant breeding is referred to as a numbers game. The experience from advanced and very successful breeding programmes in Europe and the U.S.A. shows that, normally, one or two plants out of a million or a million and a half of F_2 generation give rise to a successful crop variety (Lupton, 1984, Jensen, 1983). Since 99 per cent of all individuals in a breeding programme will eventually be discarded, it is desirable to eliminate unproductive material as early as possible, so that the limited resources are effectively utilised for exploiting more potential material. The choice of numbers to be handled in a breeding programme is made at two levels (1) the number of crosses, and (2) the number of individual plants and progenies in the segregating generations of a cross. Genetic understanding of desired characters, stage of a breeding programme

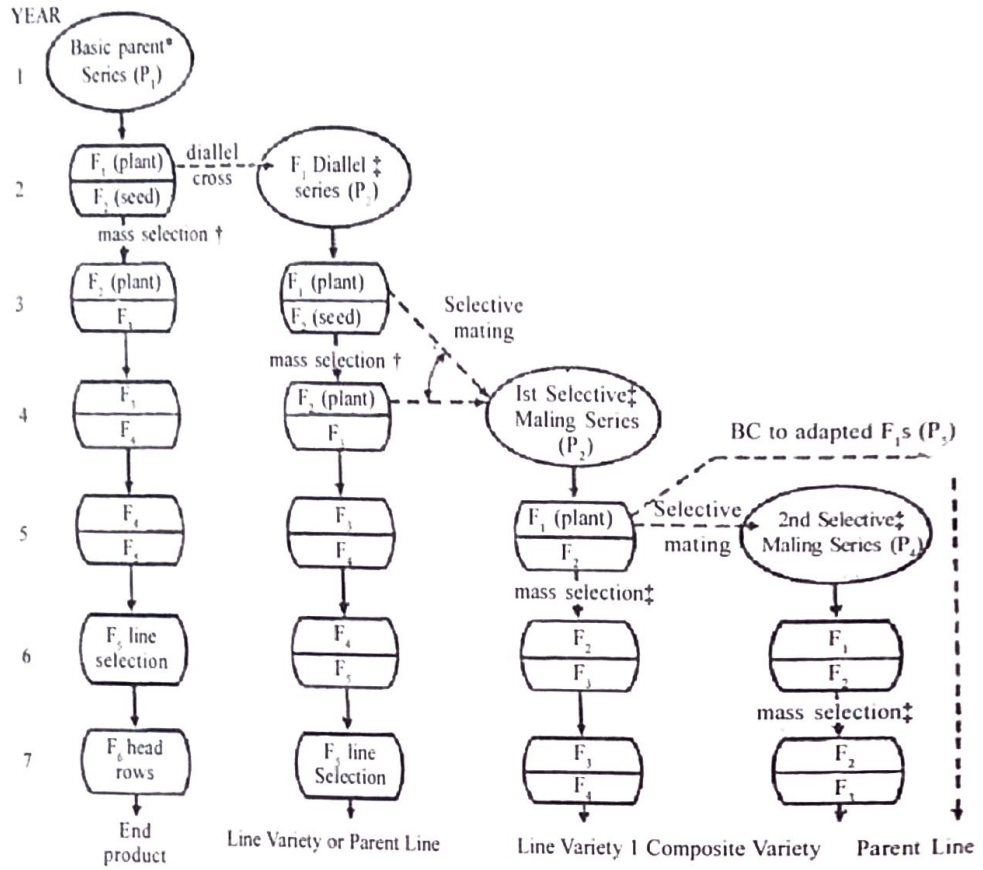


Fig. 5. Diagram of implementing steps in the diallel selective mating system for self-pollinated species (Jensen, 1970).

*Compositing optional, mass selection optional. Crosses may be individually processed through conventional system.

†Mass selection on grain and plants recommended but optional in succeeding generations,

‡Compositing of hybrid seeds mandatory, mass selection and selective mating mandatory initially.

and biometrical procedures help in attaining precision and efficiency in handling the numbers at the two levels. Nevertheless, breeder's own experience with the crop, and the necessity and urgency for a new variety play important roles in determining the approach. Some breeders emphasise on a large number of crosses, while others rely on selection in large segregating populations of a few promising crosses. In my opinion, smaller populations of a large number of crosses may be useful in case of qualitative characters governed by one or two genes, such as disease resistance. While in case of quantitative characters, such as yield and quality, selection in a large segregating population of a few promising crosses is more appropriate.

Harrington (1940), Harlan *et al.* (1940) and Immer (1941) suggested that the yield of early generation bulk could be used to identify potentially superior crosses of wheat and barley. However, results of Fowler and Heyne (1955) in wheat, Grafius *et al.* (1952) in barley, Atkins and Murphy (1949) in oats, Kalton (1948)

and Weiss et al. (1947) in soybeans did not find this approach useful for discriminating among crosses.

In recent years, with the realisation that additive type of genetic variance predominates in self-pollinated crops, interest in early generation testing has revived [Cregan and Busch (1977); Bhullar *et al.* (1977); Knott and Kumar (1975); Hamblin and Evans (1976), Saxena and Sharma (1983)]. Nass (1979) observed that crosses identified as high yielding in F_1 had significantly greater mean yields in F_4 than those of crosses that were low yielding in F_1. The high yielding crosses had three to four times as many lines yielding top 10 per cent in F_4 than did the low yielding crosses. Reviewing these studies Saxena and Sharma (1983) suggested that low yielding crosses can be safely rejected on the basis of F_1 performance. In general, the level of performance of the crosses which give low yield in the F_1 is not likely to improve substantially in later generations, except in certain very wide crosses. The probability of recovering high yielding segregates from a low yielding cross is low. However, to overcome the difficulties posed by occasional heterosis manifestation and availability of adequate seed supply for F_1 yield testing, F_2 and F_3 bulks of crosses having high F_1 yield should be tested in replicated multilocation tests.

Hamblin and Evans (1976) emphasised that apart from mean yield of cross bulks, variance should also be considered in selecting crosses for advancement. However, Hamblin (1977) in beans and Green *et al.* (1981) in pigeon peas showed that variance of individual plant yields in some of the parents was similar to those of the F_2s. Hamblin (1977) explained these results on the basis of "genotype-density competitive ability interactions" and suggested that the confounding effects of competition on cross variance could be avoided by growing the genotypes at a low density to provide a noncompetitive environment. In most breeding programmes, it may not be practical to determine the variance of cross bulks by quantitative measurement of individual traits. Instead, a visual assessment of variability in the cross bulks and an eye on the variance for characters of interest, besides mean yield of F_2 or F_3 bulks, will be useful for identifying promising crosses.

Handling of Segregation Populations

Conventionally segregating generations of a cross are handled by (1) pedigree selection, (2) backcross, and (3) bulk advance. The manner in which segregating generations are handled constitute the principal breeding methods for self-pollinated crops.

Pedigree method: In conventional pedigree method selection begins in F_2 generation, when individual plants are selected with the intent to produce the

best progenies. Hybrids between diverse parents segregate for a large number of genes and every F_2 individual is genetically different from other individuals.

Since, the probability of combining several genes in a genotype increases with the increase in the number of individuals in a F_2 population, appropriate population size becomes crucial for the success of recovering desirable genotypes. Generally, 2,000 to 5,000 plants per cross are grown in F_2 generation, depending on the availability of resources. The effective population size of 2,000 seems to be appropriate from both practical and theoretical considerations. Sneep (1977) pointed out that with 21 independent loci in a cross between two varieties, a random sample of 1,684 F_2 plants will not suffer an irreparable loss of desirable alleles. Also, Shebeskiand Evans (1973) argued that if parents differ by 25 important genes, one F_2 plant in 1,330 retains the best 25 alleles in either homozygous or heterozygous condition.

In a pedigree selection programme superior individual plants are selected in successive segregating generations and a complete record of parent progeny relationship is maintained. In F_2 generation, individual plants are selected, which in the judgment of the breeder will produce the best progeny. In F_3, first selection is made on the basis of the performance of the progeny and then the best individual plants in the progeny are selected. In subsequent generations, selection is based on: (1) performance of the family on the whole; (2) the best progeny in the family; and (3) then the best individual plants within a progeny. In a conventional breeding programme, the process is continued till F_5 or F_6 generation, when the promising progenies are bulked and replicated multilocation tests are initiated. Individuals selected in F_3 progeny constitute a family of F_4 progenies and good performance of all the progenies in a family may be more reliable than selection based on a single progeny performance alone. By F_5 or F_6 generation, most families become homozygous at most loci. Therefore, selection within families is not very effective at this stage, and selection among families should be emphasised. The pedigree record is useful for eliminating closely related families having a common ancestor in F_3 or F_4. Effectiveness of pedigree selection depends upon how truly the phenotype of the individual plant in a segregating generation reflects its genotype. Selection based on single plant yield in early segregating generations has been highly ineffective in most crops. Factors resulting in low heritability of yield in early generations include environmental variation, non-additive gene effects in heterozygous plants, and high genotype-environment interaction.

As such pedigree selection has been an excellent procedure for selecting simply inherited characters including resistance to some diseases, and highly heritable quantitative characters, including days to flowering, seed size and plant height in cereals and seeds per pod in grain legumes.

Conventional pedigree selection consists of the following steps (Fig. 6).

1) Identify a potentially good cross.

2) Grow a F_2 population of 2,000 to 5,000 plants from an individual cross by space planting for individual plant selection.

The size of the population needed will vary and depend on the number of simply inherited characters such as, disease resistance (number of diseases), plant height, flowering duration, head type and straw strength, for which selection has to be practiced, and such as the selection intensity. The objective is to save enough F_2 plants to produce the number of homozygous lines that can be handled in F_5 or F_6 generation.

3) In F_3, 200 to 500 single plant progenies should be grown to allow further selection for the highly heritable characters besides the yield of the progeny. Top 20per cent to 25 per cent progenies should be identified for selecting 5 to 10 individual plants in each of the selected progenies for advancing to F_4. Generally, more plants are selected in a progeny which appear more promising.

4) In F_4 and F_5 the process of selecting promising families and progeniesis continued.

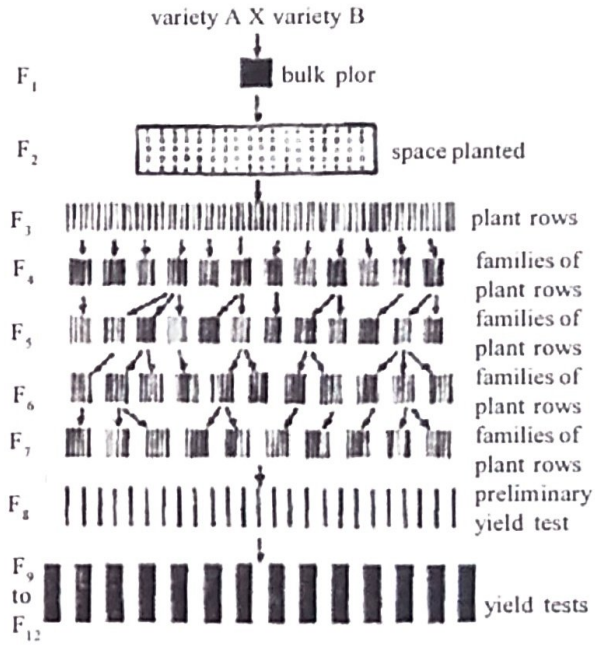


Fig. 6. Pedigree method of selection. From selected F_2 plants, progenies of 250 to 300 plants are grown in plant rows in the F_3. Superior plants from the best rows are selected and planted in families of plant rows in F_4. Selection is repeated in F_4, F_5, and F_6, with selection being made of best plants, in best rows, of best families. By F_7 families should be relatively uniform. Preliminary yield tests are planted in F_8, and yield tests are continued through the F_{12}. Various modifications of this procedure may be made. For example, after plants are selected in F_3 and F_4, remaining plants in row may be bulked and preliminary yield tests started.

5) The best 40 to 50 unrelated progenies from the F_5 generation are individually bulked for replicated yield test at one or two locations.

6) In F_6 onward, the superior progeny bulks are tested in multilocation replicated tests and only 10 to 20 per cent lines are advanced further.

The steps indicated above relate to the handling of an individual promising cross. However, a reasonably sized breeding programme deals with 10,000 to 50,000 cohorts (progenies from different crosses included for evaluation at the same time) in F_3 and ends up in 50 to 100 lines for replicated tests. Out of this, one or two may result in a variety for release.

In the pedigree system, selection of an individual genotype in the F_2 generation, and individual plants in the lines is based on visual assessment of yield and their overall agronomic worth until F_5 and F_6. Yield tests are conducted only afterwards.

It is likely that by the time yield testing begins in F_5 or later, the best genetic combinations have been lost by chance. The method involves taking notes on individual progenies. This entails considerable waste of time and resources because till rejection, a large number of poor yielding strains have to be propagated.

The following modifications have been suggested to increase the efficiency of pedigree method for identifying superior yielding lines:

1) In F_3 and F_4, when visual selection is used as a means of screening lines, intensity of selection should be relatively low (Briggs and Shebeski,1970).

2) The first selection should be made between F_2 derived lines. The final selection should be made within superior derived lines in the F_6 to F_{10} generation (Frey, 1954). This will save a lot of time and effort that is spent in conventional pedigree method. Shebeski and Evans (1973) argued that, if parents differ by 25 genes for yield, only one plant in 1,330 F_2 plants will carry all the favourable genes in homozygous or heterozygous condition. If selection is delayed until the F_4 generation, only one plant in 1.8 million is expected to carry all the favourable alleles. Thus, the frequency of superior genotypes in a population decreases rapidly with the advance of generations. Likewise, the odds of recovering superior genotypes decrease rapidly in a pedigree system when a relatively small number of plants are selected with-in lines in each succeeding generation. In case of adapted × adapted crosses, the number of yield genes by which parents differ is expected to be substantially smaller, on an average, and the frequency of plants carrying all the favourable alleles would be higher in each succeeding generation.

3) Selection should be based on both individual plants and families in F_3 and later generations. After single plants are selected, the family mean should be computed and the families with highest means should be chosen for selecting the best individual plants (Thakare and Qualset, 1978).

4) Thorough evaluation of F_2 or F_3 derived lines (F_2 and F_3 derived lines refer to a progeny derived from one F_2 or F_3 plant handled as a bulk progeny irrespective of the generation in which it is yield tested) in F_3 and F_4 generations for yielding ability and agronomic worth will improve the chances of recovering desirable high yielding types in later generations. F_3 derived lines are in common use as varieties in wheat, soybean and groundnut in theU.S.A. Since, by the fourth generation after a cross in self-pollinated crops, the non-additive gene effects are nearly dissipated (Grafius *et al.*, 1952) there is a good relationship between the performance of F_2 and F_3 derived linesand the selections made in the later generations.

Handling of three-way crosses (TC) by the pedigree method

Three-way crosses are being extensively used for incorporating genes from diverse germplasm. The basic difference between the three-way crosses and single crosses is that the F_1 of a three-way cross is similar to the F_2 of a single cross, since individual F_1plants of a three-way cross are genetically different from each other. Therefore, individual plant selection has to start in F_1 itself. For this selection to be effective, a reasonably large number of crossed seeds (300-500 seeds) is required in three-way crosses as compared to a few (10 to 15 seeds) in a single cross.

Since three-way crosses provide an opportunity of incorporating desirable genes from grand parents into the well adapted genetic complex already present in the genotype of the third parent, the TC F_1 plants should be selected as close as possible to the third parent in general appearance and maturity; and in some highly heritable attributes contributed by the grand parents. With such a selection procedure, it has been observed that TC F_2 progenies are strikingly uniform, even though they are similar to F_3 progenies in their constitution. Thorne and Fehr (1970) indicated substantial gene fixation for yield in the F_2 generation of soybean crosses. TC F_2 derived progenies can be successfully tested for yielding ability in replicated trials or in observation nurseries; alternatively, they can be handled as per conventional pedigree selection procedure.

Pedigree selection is most effective in quickly establishing stable lines for highly heritable characters and allows maximum opportunity to a plant breeder to exercise his skill and experience to improve a particular crop. Its main disadvantage is that an individual breeder can handle only limited material.

Backercoss method

Breeders of early twentieth century engaged in the development of disease resistant varieties observed that pure line selections with genes for resistance from intra- or inter-specific hybridisation were inferior to the generally acceptable superior parent in yield or quality characteristics. To overcome this problem, Harlan and Pope (1922) suggested the backcross method by which an undesirable allele at a particular locus is replaced by the desirable allele in otherwise elite variety. In other words, backcross procedure conserves all good characteristics of a popular adapted variety and incorporates a desirable character from another variety. To start with, the backcross method was mainly used for developing disease resistant varieties of barley and wheat particularly against rust of wheat in Australia and the U.S.A.

Briggs and Allard (1953) identified the following prerequisites for the success of a backcrossing programme:

1. Existence of a good recurrent parent variety which requires improvement in some qualitatively inherited character or a quantitative character with high heritability.
2. High expressivity of the character under transfer through several backcrosses in the genetic background of the recurrent parent.
3. Simple testing technique for detecting the presence of the character under transfer.
4. Recovery of the recurrent genotype in a reasonable number of backcross generations.

The procedure of backcross programme varies in operational details depending on the genetic nature of the character under transfer that is simple or multiple gene inheritance and dominance and the recessive nature of the gene. The generalised procedure of a backcross programme is as under:

1. Two selected parents are crossed. One parent is an adapted productive variety, which needs to be improved for a character available in the other variety.
2. The F_1 of the cross is again crossed to the adapted productive parent known as the recurrent parent. The other parent, called the non-recurrent or donor parent, in the cross, provides the desirable allele for the character under transfer.
3. After each backcross selection is made for the character from the donor parent and the individual plants carrying that character are successively backcrossed five to six times to the recurrent parent. The number of backcrosses may vary from one to six depending on how closely the breeder wants to recover the genotype of the recurrent parent.

Selection for a desirable allele in a backcross generation is easier than in a F_2 population of a cross since the proportion of desirable homozygous allele is 1/4 (F_2 1/4 AA+l/2Aa+l/4aa) as compared to 1/2 (BC1 1/2AA+ 1/2Aa) in the backcross generation.

In the backcrossing programme homozygosity is attained at the same rate as with selfing according to the formula

$$\left(\frac{2^m - 1}{2^m}\right)^n$$

where, m = number of generations of backcrossing plus the original cross

n = number of gene pairs involved in the transfer.

For example, after four backcrosses reconstitution of recurrent parent genotype differing in a single pair of genes could be calculated as follows:

$$\left(\frac{2^m - 1}{2^m}\right)^1 = \frac{31}{32} = 93.75 \text{ per cent}$$

With every backcross to the recurrent parent the proportion of undesirable genes from the donor parent is reduced to one half. The elimination of undesirable genes from the donor parent is influenced by linkage. Nevertheless, eliminating the undesirable linked allele from the donor parent are more in a backcross programme than through selfing in a pedigree selection programme (Table 1), if there is no selection against the undesirable linked allele selection can be practiced against the undesirable linked allele, the chances of getting desirable recombinants are more under selfing rossing since under selfing recombination can take place in both the male and the female gametes. In case of backcross there is no recombination in the recurrent parent. The backcross procedure is advantageous in the sense that even without any selection against the other undedesirable gene seventually, the genotype of the recurrent parent is constituted. In addition only the desirable gene from the donor parent, which is of course maintained in the population by selection, is added.

Table 1. Effect of linkage on the probability of eliminating an undesirable gene (b) linked to a desirable gene (A)

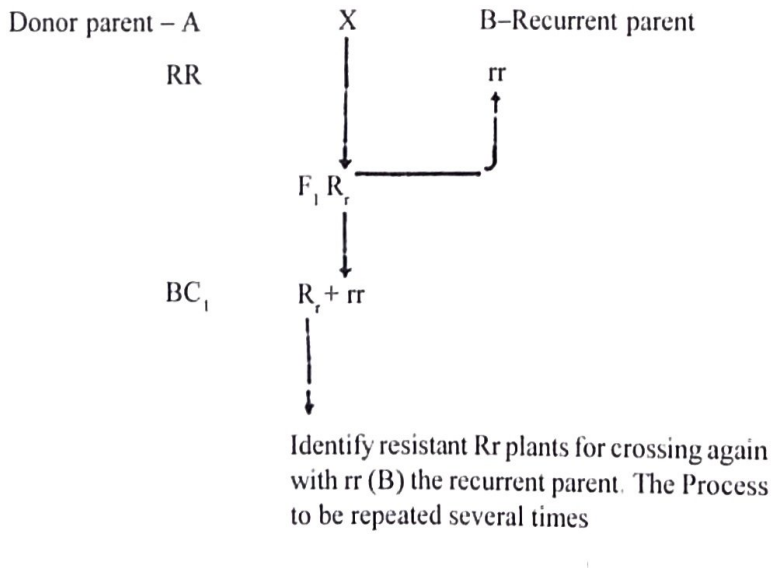
	Probability that the undesirablegene will be eliminated	
Recombination fraction	With five backcrosses	With selfing
0.50	0.98	0.50
0.20	0.74	0.20
0.10	0.47	0.10
0.02	0.11	0.02
0.01	0.06	0.01
0.001	0.006	0.001

It is assumed that selection is practiced for A but is not practiced against b.

Backcross procedures for different genetic situations are modified to suit the detection of the character under transfer so that plants can be easily identified to which a dose of backcross can be given. The procedures for different situations are described below.

1) Transfer of characters controlled by a dominant or partially dominant allele

Identification of dominant and partially dominant alleles is easy. Individuals carrying them can be easily identified in heterozygous conditions and backcrossing on such heterozygous individuals can be continued. If the character under transfer cannot be identified morphologically, such as disease resistance, the heterozygous plants (Rr) carrying the resistance gene (R) can be distinguished from the susceptible plants (rr) by inoculating with the disease pathogen. Then, only the resistant plants are crossed with the recurrent parent, as shown:



2) Transfer of a recessive allele

If the allele under transfer is recessive (ms) the progeny from each of the backcross would segregate into two genotypes (Ms Ms) and (Ms ms). Since the heterozygote which carries the desired allele (ms) cannot be identified, it is necessary to self the progeny to find the homozygous (ms ms) plants before backcrossing to the recurrent parent. Another possible approach is to backcross both the homozygous (Ms Ms) and heterozygous (Ms ms) plants to the recurrent parent and at the same time self each plant and test the selfed progenies for heterozygosity. The backcross progeny from the plants that prove to be heterozygous is kept, and the backcross progeny from the homozygous (Ms Ms) is discarded (Fig. 7).

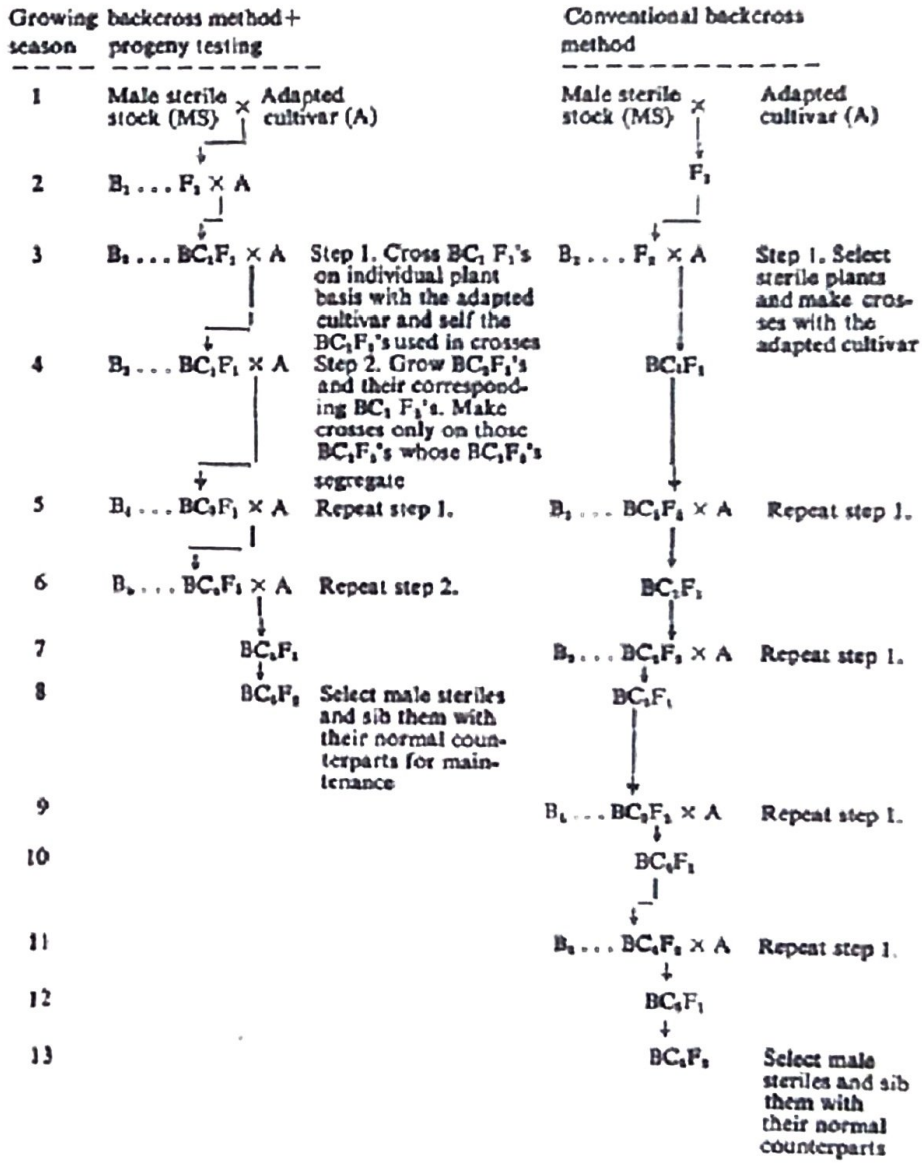
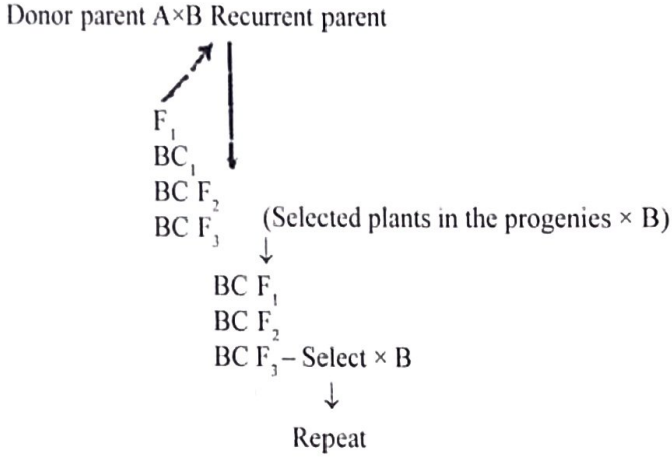

Growing season
backcross method + progeny testing
Conventional backcross method
1
Male sterile stock (MS) × Adapted cultivar (A)
Male sterile stock (MS) × Adapted cultivar (A)
2
B1 . . . F1 × A
F1
3
B2 . . . BC1F1 × A
Step 1. Cross BC1 F1's on individual plant basis with the adapted cultivar and self the BC1F1's used in crosses
B2 . . . F2 × A
Step 1. Select sterile plants and make crosses with the adapted cultivar
4
B3 . . . BC1F1 × A
Step 2. Grow BC2F1's and their corresponding BC1 F1's. Make crosses only on those BC1F1's whose BC2F1's segregate
BC1F1
5
B4 . . . BC2F1 × A
Repeat step 1.
B3 . . . BC1F2 × A
Repeat step 1.
6
B5 . . . BC2F1 × A
Repeat step 2.
BC1F1
7
BC3F1
B4 . . . BC2F2 × A
Repeat step 1.
8
BC3F2
Select male steriles and sib them with their normal counterparts for maintenance
BC2F1
9
B5 . . . BC3F2 × A
Repeat step 1.
10
BC4F1
11
B6 . . . BC4F2 × A
Repeat step 1.
12
BC4F1
13
BC4F2
Select male steriles and sib them with their normal counterparts

Fig. 7. Comparison of backcross method coupled with progeny testing and conventional backcross method.

3) *Transfer of a quantitative character*

A quantitative character is controlled by several genes and it is difficult to identify the desired plant in backcross F_1 generation. In this situation it is desirable to advance the progenies to backcross F_2 and F_3 generations and backcrossing is alternated with progeny testing as follows:



The number of backcrosses will depend on the extent of recovery of the desirable level of the quantitative character under transfer. It is generally accepted that three to four backcrosses are adequate and at this level transgressive segregants, combining the desirable genes from both the parents, may also occur. This will be an advantage over reconstituting the genotype of the recurrent parent only. It may be pointed out that it is easy to transfer a quantitative character having high heritability than one with low heritability. The problem of low heritability can be tackled to some extent by increasing the number of plants in BCF_2 and BCF_3 generations.

Smith (1966) suggested that backcrossing should be introduced in F_3 and F_4 selected lines rather than in the early stages in order to have bonus characters from both the recurrent and the donor parents and recover transgressive segregants.

4) Transfer of more than one character by backcrossing

If two or more characters are to be added to a recurrent variety, it would be desirable to incorporate individual characters in separate backcross programmes involving the same recurrent parent. At the end of the backcross programmes for individual characters, the backcross derived lines be merged into a single line.

Generally, backcrosses are used for the following purposes:

1. Transfer of disease resistance.
2. Incorporation of exotic germplasm (Lawrence and Frey, 1975).
3. Incorporation of genes for quality such as protein content.
4. Development of multiline varieties carrying resistance genes for different races.
5. Development of varieties with multiple disease resistance.
6. Incorporation of male sterility and restorer genes for hybrid varieties.

Bulk method of breeding

The bulk method of handling segregating generations of across or composite cross population refers to a procedure, where segregating generations are advanced as a pool of genotypes without any artificial selection. The method was first used by Nilsson-Ehle of Sweden for handling segregating generations of a hybrid made to combine winter hardiness of the Squarehead variety with the high yield of the Stand-up variety of winter wheat. As indicated by Allard (1960) Nilsson-Ehle had well appreciated and applied the important features of the bulk population method as under:

1. Natural selection eliminates poorly adapted low yielding types from the population. However, he hastened the process of increasing the winterhardy genotypes by discarding plants which suffered winter damage but would have otherwise produced some seeds.
2. Growing of a large population was essential for increasing the chances of recovering high yielding types.
3. The bulk method was the easiest for handling a large population as no record keeping and harvesting of individual plants was required.
4. Advancing generations by bulking will increase the homozygosity of individual plants in self-pollinated crops. Selections made after a few generations of bulking are likely to breed true.

Harlan *et al.* (1940) proposed the bulk method to handle a large number of crosses and extensive genetic variability generated in case of composite crosses involving several parents. The method involved yield testing of F_2 bulks from a number of crosses and discarding the poor yielding crosses.

The selected crosses are then advanced by growing a large F_2 population (5,000 to 10,000 plants) at normal plant density and harvesting the material in bulk at maturity for planting the harvested seeds in a similar size plot in the following season. This process is repeated as many times as desired by the breeder. Normally by F_6 generation, most of the plants in the bulk population will reach a high level of homozygosity. In F_7 and F_8 single plant progenies are selected for yield tests (Fig. 8).

Since selection of individual plants in F_2 and F_3 for yield is ineffective (Sakai, 1954; McGinnis and Shebeski, 1968; Knott, 1972; De Pauw and Shebeski, 1973; and Knott and Kumar 1975) bulk handling of segregating generations seems to be the easiest procedure for propagating large populations of self-pollinating plants from crosses to establish homozygous lines.

The success of the procedure depends on:

1) Yield of different genotypes which is affected little by genotype × environment interaction from one generation to another. This means that high yielding genotypes will also yield high in the next generation, so that, their frequency in

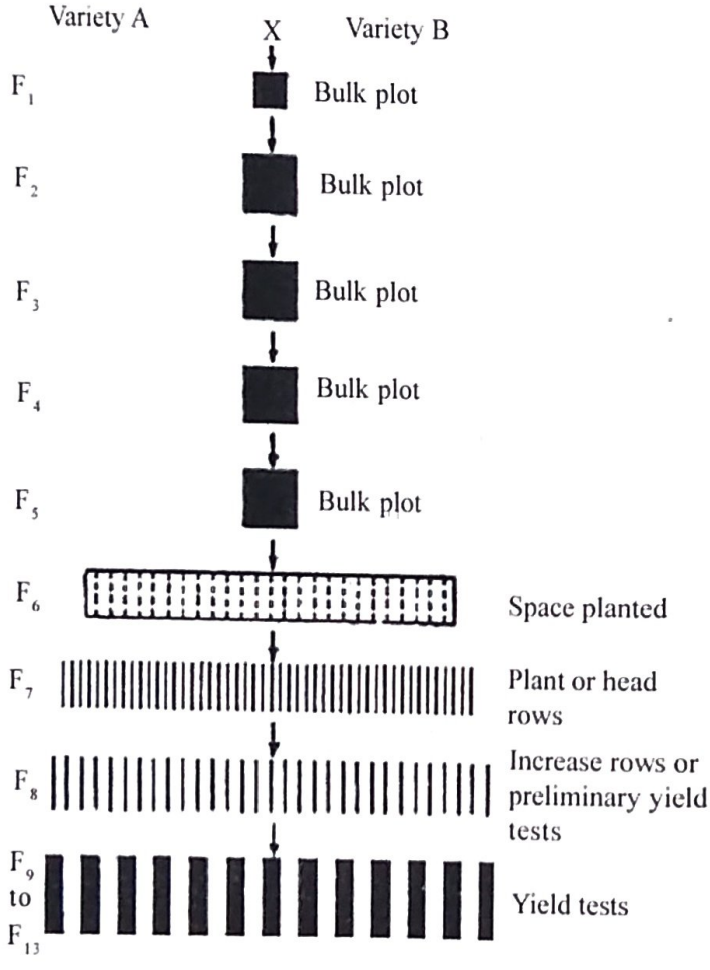


Fig. 8. Bulk population method of selection. The progeny of the cross is grown in a bulk planting through the F_6 generation. In F_6 the progeny is space-planted. Plant or head selections are made and grown in plant or head rows in F_7. Superior rows are selected and grown in increase rows or preliminary yield test in F_8. Superior strains are grown in yield tests in F_9 through F_{13}. Various modifications of this procedure may be made. For example, selection may start as early as F_3 or F_4, with lines having a superior yield being purified in later generations, or bulk plots may be replicated and harvested for yield, and entire crosses discarded on the basis of the yield of the bulk plots.

the population increases year after year. Bulk populations do not change much with regard to the frequency of high yielding genotypes because variations of genotype × environment interaction from one year to another favour different genotypes in different years and keep the population in an equilibrium. Tucker and Harding (1974) studied the effect of environment on seed yield in bulk populations of lima beans and observed considerable differential natural selection between locations in the same production region. If this environmental variation between locations is indicative of possible environmental variation at a location from year to year then natural selection may not be effective in improving the frequency of high yielding types in a bulk population.

2) Relationship of competitive ability and agronomic superiority of the genotypes in the population. Evidence for competitive ability of genotypes and their performance comes from (a) studies on varietal survival in pure line mixtures; and (b) survival of different plant types in hybrid-derived populations.

Studies on varietal mixtures by Suneson (1949) in barley and Mumaw and Weber (1957) in soybean indicated that the ability of a genotype to survive in a varietal mixture, and its productivity in a pure stand, were not related. Also, Frey (1967); Reyes and Frey (1967) and Tiyawalee and Frey (1970) did not observe any effect of natural selection in bulk hybrid populations of oats over several generations for characters such as 100-seed weight, heading date, plant height and resistance to crown rust. On the other hand, Jennings and Herrera (1968) found that rapid natural selection occurred in bulk rice populations derived from hybridisation of tall and dwarf rices. In bulk population, tall plants had a reproductive value of 1.0 against only 0.68 for the dwarf plants, in spite of the fact that the derived dwarf lines in F_6 yielded 2.0 m t/ha more than their tall counterparts. Similarly, Khalifa and Qualset (1974 only, 1975) observed that natural selection favoured the relatively low yielding tall segregates in a bulk population of tall and dwarf wheat hybrids. They suggested that bulk populations can be very useful sources of high yielding adapted lines, if the bulks are so managed as to avoid the obviously undesirable competitive effects.

However, Suneson (1956) showed that bulk populations from multiple crosses of barley increased in yield with advancing generations extending up to 28 generations. He observed that a population handled as a bulk for a few generations changes very slowly in its population structure unless there is a high degree of selection pressure. In case of quantitatively inherited characters such as yield significant shift in the mean usually does not occur until about the 15th generation. Suneson and Stevens (1953) also observed the survival of alternative alleles at five loci in a barley-bulk population composite cross I and concluded that in this population agronomically poor types are also poor competitors.

Generally, wide differences in growth habit and plant phenology are associated with negative relationship between the competitive ability of individual plants and productivity under commercial high input crop management as observed in the case of rice and wheat crosses involving tall and dwarf plant types. Bulk populations from crosses involving parents with diverse phenology can be effectively handled by dividing the segregating bulk into two or three sub-populations representing the different plant phenologies. Competition in such a situation will be between genotypes of similar phenologies and, therefore, comparatively high yielding genotypes will predominate in the bulks of each phenology. Empig and Fehr (1971) observed that maturity group bulks were quite effective in avoiding undesirable effects of natural selection in segregating generations of soybean crosses and could maintain a high degree of variability for different plant characters.

The effects of natural selection in bulk populations can be briefly summarised as under:

1. Genotypes with high competitive ability may not necessarily be superior yielding under commercial cultivation with high input management conditions, particularly from crosses between adapted × exotic germplasm. However, in adapted × adapted crosses agronomically poor types are also poor competitors.
2. Morphological uniformity of bulk population increases with advancing generations, but considerable variability is maintained in the population even after 20 or more generations of natural selection.
3. Yielding ability of bulk population increases with continued bulking, particularly in case of adapted × exotic crosses. Proportion of superior selections increases steadily over long periods of bulk handling.
4. Natural selection rapidly adjusts the characters such as maturity, plant height and adaptation.

Natural selection is most effective in stabilising the bulk population for characteristics directly responsible for adaptation of the genotypes in a given environment and management situation. Artificial selection is very useful in bulk populations to eliminate obviously undesirable types such as tall indeterminate plants, certain seed colour, plants susceptible to diseases, and stresses such as drought or frost. For subtle differences, natural selection may be more effective than that imposed by the plant breeder. Since, natural selection is more effective in discriminating between fixed homozygous types than the heterozygotes in the early generations, long periods of bulk handling is essential for character fixation, because homozygous types are fixed by F_6 generation (Jain, 1961; Allard and Jain, 1962).

Bulk method as a plant breeding procedure is not very attractive to the plant breeders as it requires a long time to fix the genotypes and identify the desirable ones. The method is most advantageous for obtaining homozygous lines with the minimum of effort and expense, and for maintaining large variability over an extended period during which selection of desirable adapted types can be made when observed (Suneson and Wiebe, 1962). The bulk method is most effective in crops like cereals and grain legumes, which are planted in thick stands and where individual plant selection is not effective for quantitative characters. In crops such as vegetables and fruits, the bulk population method does not have much advantage.

Modifications of bulk method

1) Mass-pedigree

2) Single seed descent method (SSD)

1) *Mass-pedigree:* Harrington (1937) proposed the mass-pedigree method of handling segregating generations as a means of overcoming the limitations of pedigree selection. Characters such as straw strength, resistance to shattering, earliness, and disease resistance can not be handled by pedigree selection in dry years, when the environmental conditions are not favourable for their expression. Harrington suggested that to avoid the waste of effort and expense of ineffective pedigree selection the segregating material may be carried in bulk till favourable conditions prevail and effective single plant selections can be made. Here upon the selections are handled as single plant progenies. If the environmental conditions are not favourable for distinguishing between progenies in a particular year, they are harvested as progeny bulks rather than exercising selection between and within progenies.

2) *Single seed descent method (SSD):* The single seed descent method of advancing segregating generations of self-pollinated crops was suggested by Goulden (1939). The concept behind this procedure is to attain a reasonable level of homozygosity for characters of low heritability such as yield, which are difficult to select for in the early generations before they are evaluated. This avoids the problem of natural selection favouring more competitive agronomically poor types in the bulk population breeding method. Grafius (1965) and Brim (1966) modified the method and suggested advancing each F_2 plant in the population by single seed descent (Fig. 9).

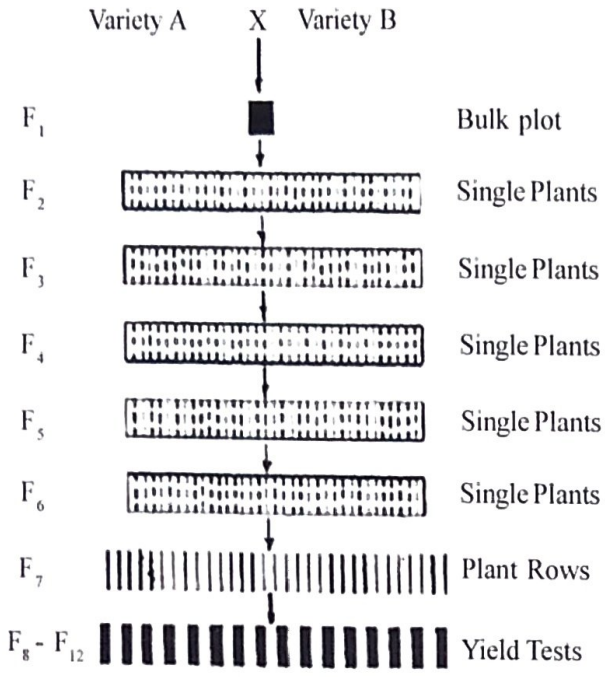


Fig. 9. Single-seed-descent method of selection. Seeds harvested from F_1 plants are space planted in F_5. A single seed harvested from each F_2 plant is used to plant the F_3. Succeeding generations through the F_6 are likewise planted from single seeds harvested from each plant grown in the preceding generation. In the F_6 generation plants are harvested and a progeny row grown in the F_7. Superior F_7 plant rows are harvested and yield tests started in the F_8 generation. The objective is to maintain the descendants from a maximum number of F_2 plants during the segregating generations.

In the F_2 and succeeding generations only one seed is used from each plant in the population selected for advancing to the next generation. For practical reasons, a single pod (two to three seeds) is taken from each plant, but only one plant from the pod is retained for advancing to the next generation. When the desired level of inbreeding is attained, each progeny, which traces back to a F_2 plant, is maintained in bulk.

Brim (1966) listed the following advantages for the system in comparison with the pedigree system of breeding:

1. Less space is required per generation since single plant hills are grown for each F_2 plant compared to the progeny rows for selected plants in case of pedigree selection. In early stages close spacings can be used. Wide spacing is necessary only in the last generation for producing sufficient seed for testing individual plant progenies.
2. Time and labour spent in harvesting is much less.
3. Record keeping is considerably reduced.
4. Selection for high heritability can be effectively exercised on the basis of individual plants.

5) Several generations can be grown per year by using green house and off-season nursery facilities, since no selection for agronomic characters is applied. Grafius (1965) pointed out that generation turnover can be extremely rapid in low-nutrient, continuous light environment, with each plant producing only a few seeds.
6) Less effort is spent in obtaining homozygous lines.

The method has been evaluated by Kaufmann (1971) in oats, Boerma and Cooper (1973), and Empig and Fehr (1971) in soybean, and Thean and Qualset (1975), and Knott and Kumar (1975) in wheat. They all observed that the SSD method is quite convenient and time-saving and is able to maintain considerable variability. It has considerable merit as a procedure for advancing bulk hybrid generations and recovering agronomically desirable genotypes in certain situations.

However, Frey (1968) observed that the SSD method provides no opportunity for differential reproductivity of parental plants and reproductively inferior genotypes are carried along with superior genotypes. He suggested that the SSD method should be used only in cases where little relationship is expected between the reproductive values of genotypes in a bulk population and their agronomic values as individual genotypes. He listed the following disadvantages of the method:

1. The identity of superior F_2 plant is lost.
2. The system provides no opportunity by which a superior genotype identified from the bulk could be isolated and used as stop-gap arrangement in F_4 or F_5.
3. The system is useful only when several short life cycles under artificial conditions can be advanced in a year.
4. The system provides no opportunity for recurring phenotypic selection in early generations.

Testing and evaluation

Evaluation of recombinant genotypes after hybridisation depends on the breeding method adopted for achieving a particular breeding objective. In the case of pedigree selection, the first phase of evaluation starts from F_2 onwards and involves segregating individual plants and progenies; the second phase starting from F_5 or F_6 deals with homozygous individual plant progenies. In backcross and bulk population methods, evaluation of recombinant genotypes is delayed till a high level of homozygosity is achieved towards the end of segregating generations as discussed earlier and selection is practiced on progeny performance between homozygous lines.

Since the evaluation procedure for advance homozygous lines is common to all the breeding methods aiming at producing pure line varieties, details of evaluation and testing of genotypes in a pedigree selection programme are discussed to cover the general approach for self-pollinating crops.

Evaluation of genotypes in a pedigree selection programme can be conveniently classified into three stages:

1. Evaluation of genotypes during segregating generations.
2. Preliminary evaluation of homozygous advance lines.
3. Final evaluation of promising lines for performance and adaptation in regional tests.

Evaluation during segregating generations

During segregating generations (F_2 to F_5), individual plants and single plant progeny rows are evaluated in one environment and selected for highly heritable characteristics, such as, disease resistance, maturity, plant height and type, seed size and colour.

The initial line evaluation phase begins with the F_3 generation, when individual plant families and progenies are established. A good size breeding programme may have 1,000 to 20,000 plant progenies (Cohorts-progenies from different crosses in the same generation entering at the same time in the test nursery) in F_3 generation depending on the stringency of selection for qualitative and highly heritable characters indicated earlier. In F_3, normally 10 to 15 per cent progenies are selected for advancing to the next generation. The process of family, progeny within a family and individual plant selection is continued till F_5 or F_6 generation with selection intensity of 25 to 30 per cent in each generation. It is important to discard inferior lines. Once a reasonable number of lines (around 50 to 100) is reached they are tested for yield and quality attributes in an appropriate preliminary yield trial.

For evaluation of lines in early generations (F_2 to F_4), breeders rely on their own experience and knowledge of plant characteristics, such as, plant type and yield components associated with good agronomic performance. The evaluation is mainly based on breeders visual judgement popularly known as the "Eye ball index" which constitutes the art of plant breeding.

Jensen (1983) emphasised the importance of visual selection in early generations by stating that "since 99 per cent of all individuals in a breeding programme will eventually be discarded, it is a good strategy to keep the design as simple as possible so as not to waste effort on nonpay off areas." He pointed out that with 15 per cent selection on visual basis, only 1,500 rows need to be harvested

as against the alternative of harvesting the entire nursery of 10,000 and proceeding with analysis of yield, 1,000 kernel weight, and so forth.

He suggested that a decision about the value of a line in a plant breeding programme should be reduced to two questions: (1) Assuming all unknowns to be favourable, does the line have the potential to be a new cultivar? (2) Is it wanted for any other reason, for example as a parent for further crossing? If the answer to both is 'no', there is no reason to keep the line. If the answer to either is 'Yes' or "I do not know" the line should be kept through another season. In two to three years, sufficient observation and research data are accumulated to decide about retaining or rejecting the line.

The personal judgement of a plant breeder about the agronomic utility of a particular line is no doubt valuable, but there is enough evidence to show that the decision to select or reject for characters of low heritability, such as, yield has to be based on quantitative measurements having a minimum influence of genotype × environment interaction. This is particularly important in crosses involving adapted × adapted parents where heritable differences are not marked. Briggs and Shebeski (1970) and Stuthman and Steidl (1976) have observed, in wheat and oats respectively, that visual selection for yield in F_3 was not reliable. Since in the early generations a large number of lines (1,000 to 2,000) need to be evaluated, it is practically impossible to have adequate environmental control in the test field and make the necessary measurements. Therefore, for the sake of convenience, most breeders postpone the evaluation of the lines for yield and quality to later generations that is F_6, when sufficient homozygosity of the genotypes has been attained and the number of lines is reduced to a manageable level for yield tests. Besides the reduction of lines, it is well recognised that it is easy to identify differences between homozygous fixed lines than between heterozygous segregating lines. However, Shebeski and Evans (1973) argued that "the most critical generation of evaluation is F_3, because it is easy to lose the potential of the best F_2 plants by F_3. Though we recognise the need for wide range adaptability in our varieties and in our testing programme we replicate and grow the advanced generations at the number of locations for several years, however, there is little effort to make selection on the same basis in the early generations."

A number of approaches for evaluating the early generation lines in unreplicated plots have been proposed and are being tried by the breeders.

1) Intermittent check plots of a standard variety

The use of intermittent check plots provides a good measure of the soil fertility or the micro-environmental conditions for comparing the yielding ability of an adjacent plot. Briggs and Shebeski (1968) reported that in different wheat

breeding programmes, the frequency of control plots varies from 1 in 200 plots to one every third plot, with an average of one control per 50 plots. They observed that frequent controls are essential for efficient selection for yield in unreplicated early generation nurseries. The pedigree-wheat selection nurseries at the university of Manitoba in Canada grow a control plot adjacent to every plot of breeding material. The yield of each experimental plot is expressed in terms of the yield of its adjacent control. The frequency of control plots varies from programme to programme depending on the number of lines to be evaluated, resources available, management conditions or soil heterogeneity in the nursery. Normally, in early generations the frequency of control plots is lower (5th to 10th plot) than in advance generations (3rd or 5th plot).

2) Moving means method

Knott (1927) and Townley-Smith and Hurd (1973) adapted the moving average method wherein the mean of a number of adjacent plots, excluding the plot in question, is used as an environmental index. The performance of a genotype is expressed as the difference between it and the moving mean, or as the percentage of the moving mean. Knott (1972) and Mak *et al.* (1978) observed that intermittent check plots and moving means gave similar control of experimental error, but from the practical consideration of accommodating large number of test lines in a given land area, moving means method has an advantage. In this method no plots are wasted for accommodating the check entries, which may require as much as 1/3 of the test field.

3) Augmented field plot design

Federer (1956) proposed the augmented field plot design to circumvent the difficulty of drawing valid statistical conclusions from unreplicated line tests in early generations. He suggested to include four to six control varieties for which enough material is available and to repeat them several times in a standard design. Each repetition of the control lines is embedded in a block and the test lines are assigned to plots that are not allocated to controls. Estimation of block effects and plot error is done only with respect to control lines. The estimated block effects are used to adjust the observed values of the test lines and the error is used to test the significance of lines differences.

Recently, Lin and Poushinsky (1983) have proposed a modification of the augmented design. They suggested that the layout of the plots should be structured in a split plot design, where the whole plots can be laid out in any standard design, but the arrangements of subplots are always 3×3 with the centre point used as a control (Fig. 10). The shape of a subplot needs to be square or nearly square, so that the distance between the centre plot and its

surrounding eight test plots is relatively uniform. The control lines are assigned to the control plots according to the design specification for the whole plots, and the test lines are assigned randomly to the non-control plots. The number of control plots in this design is approximately 13 to 17 per cent of the total number of plots.

Evaluation of advance homozygous lines

The surviving advance lines attain a reasonable level of homozygosity by F_5 and F_6 and, therefore, reflect their genotypic potential. If the line is a superior performer it can be identified as a variety. The aim of evaluating these lines is to find the highest yielders adapted to the general area of production. Since, field trials are expensive in terms of person-hours, supplies and land area, it is desirable that cost effective optimum testing plan is adopted. Finney (1958) has discussed the subject in detail and has concluded that increased yield from selected varieties can be largely achieved in two or three stages. Additional gains from each subsequent stage in the programme will be small.

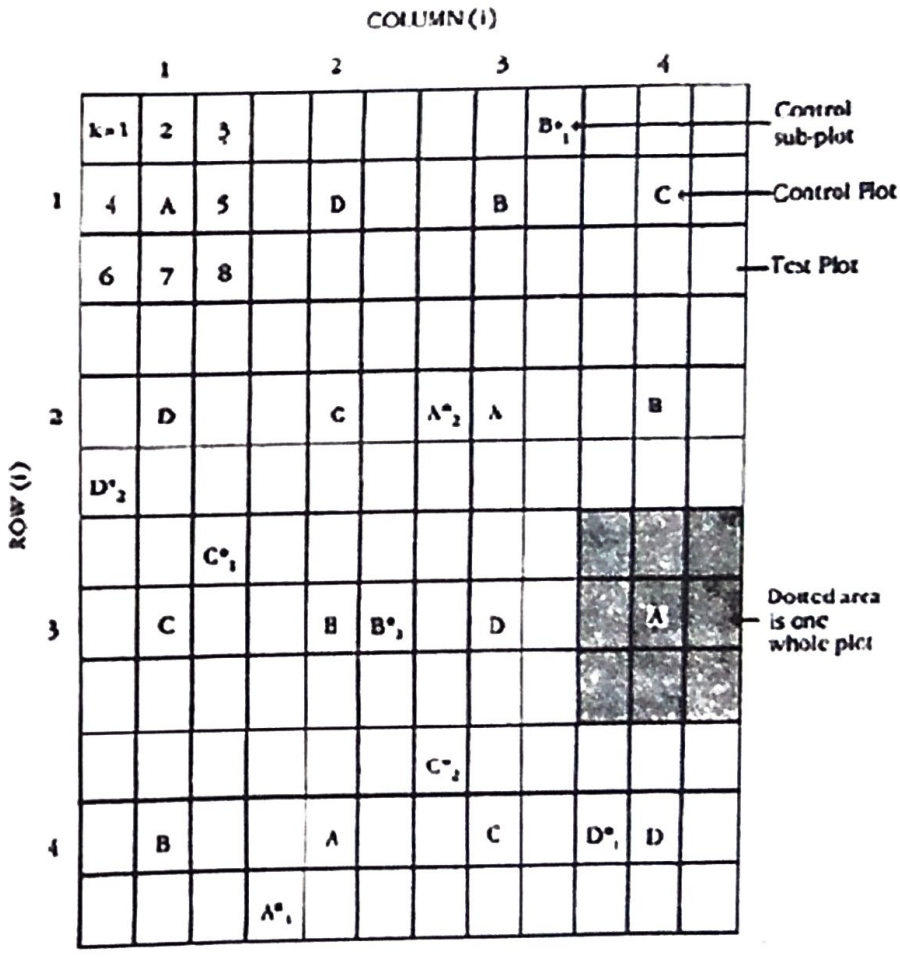


Fig. 10. Field layout for one 4 × 4 Latin square

In general, in a two stage testing programme, the testing of all lines in one environment and selecting lines for retesting in a number of environments are more efficient than testing all lines in one or two environments. Hanson and Brim (1963) discussed optimum allocation of test material for two stage testing in soybean and made the following recommendations:

1. If one good test site is available, where extreme stress conditions can be avoided by use of irrigation etc., all the lines should be grown therein in two or preferably three replications.
2. Select the top yielding phenotypes, and retest at as many sites as available, say four or five.
3. Alternatively, grow all the lines at two locations in two replications at stage 1 and retest the selected top yielding group at four or five sites. Use of two locations for the first stage test provides an insurance against abnormal conditions or seasonal hazard.

Finney (1958) suggested that when the few of the best are to be chosen from a large number of new varieties, statistical significance should not be a criterion of selection, because there will be difficulties in ranking the varieties in order. The right procedure is simply to select the best and discard the remainder without regard to significance. Trials over several seasons and rejection of poor performers in each season will leave only the best performers.

Stability of performance

The mean yield of a variety, the most common description of a genotype's performance, is subject to a high degree of genotype × environment (GE) interaction and therefore is inadequate for making a decision for release of a variety for general cultivation. It does not indicate the stability of performance, which is a function of a genotype of the variety and its population structure and constitution (multiline varieties bulk populations or composite varieties and F_3 or F_4 derived lines varieties).

Generally, a combined analysis of variance for multilocation trials conducted for at least three years is used to identify the existence of GE interac-tion. Sprague and Federer (1951) described the procedure to estimate the different variance components. In recent years, a number of methods have been proposed to identify superior stable genotypes in the presence of GE interaction. The different statistical procedures have been reviewed by Free-man (1973). Some commonly used and relatively simple approaches are mentioned below.

1. *Regression.* This method, where regression of yields of the separate varieties on the mean yield of all varieties is calculated, was first proposed by Yates and Cochran (1938). Finlay and Wilkinson (1963) rediscovered it and emphasised the usefulness of the method to determine the adaptation

of crop varieties. Varieties with high mean and low regression value are considered to be desirable.

2. *Deviation from regression.* Eberhart and Russell (1966) suggested that regression was a response index and that the mean square for deviations from regression provided the real index of a variety's stability of production. A variety with high mean, regression value near one, and with least deviation from the regression value is considered to be desirable.

3. *Mean and covariance (CV).* Francis and Kannenberg (1978) argued that the term stable genotype has often been used to describe a genotype that has constant performance over environments. Such genotypes, however, usually have below average yields (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966). They observed that varieties responsive to environments have higher variance than the non-responsive varieties. A variety responsive to environment is not necessarily unstable and is more desirable. What is required is a measure of consistency of yield from one environment to another. The Mean-CV method suggested by Francis and Kennenberg consisted of plotting the mean yield of a variety across the locations against the coefficient of variation (CV) for the mean yield (Fig. 11). By this method, varieties can be divided into four groups:

   Group I — high yield, small variation.

   Group II — high yield, large variation.

   Group III — low yield, small variation.

   Group IV — low yield, large variation.

   Group I varieties are the most desirable since they meet the requirement of a stable genotype in having high and consistent performance. It is more practical to characterise the genotypes on a group basis rather than individually. It is a simple descriptive method for grouping a large number of genotypes from yield data collected over several environments.

4. *Range in productivity:* Langer *et al.* (1979) suggested the use of range (Rl) representing the extreme yields for a variety in all environments and (R2), yields for the same variety in the poorest and best environments. They observed that the correlation of regression response index (b) with Rl, pooled across variety sets, was 0.90 and highly significant. This indicates that genotypes could be screened quite accurately for regression response indexes of Finlayand Wilkinson (1963) simply by utilising the ranges in variety means. To estimate R2 only two, fairly extreme, environments would be required and, therefore, is convenient from the practical point of view. Rl provides a more accurate estimation of production response than does R2. The method is simple and can be conveniently employed in small breeding programmes lacking extensive computation facilities.

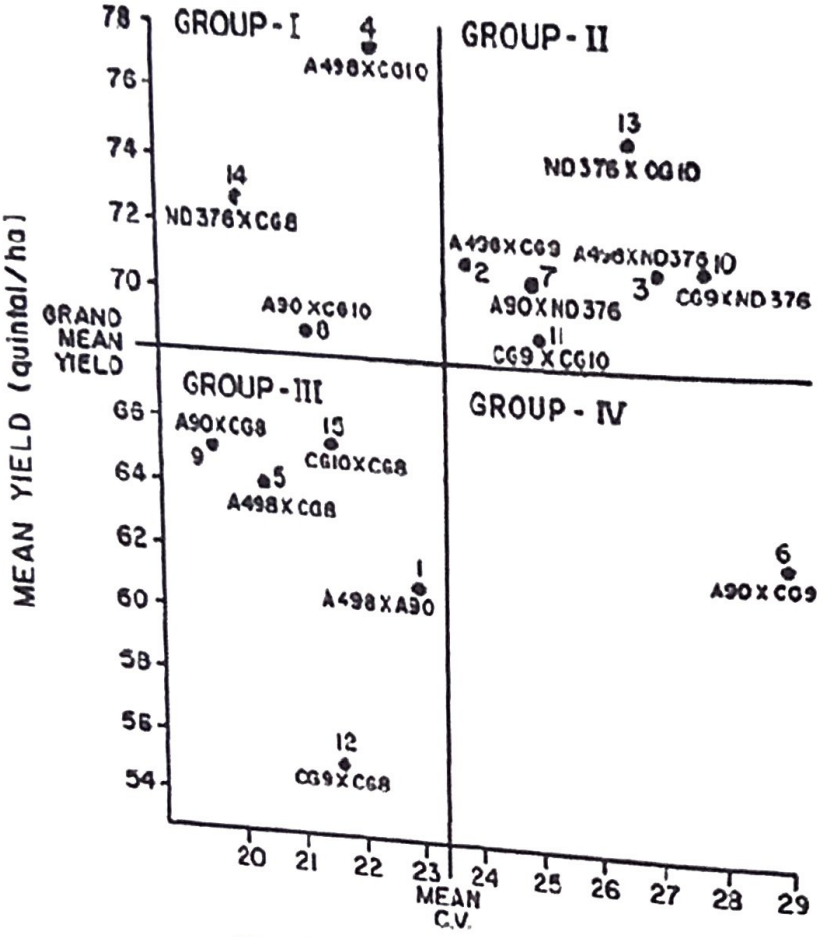


Fig. 11. Mean yield plotted against CV from data collected on 15 hybrids in 16 environments. Francis and Kannenberg (1978).

5. *Floating checks.* Generally a well known variety is used as a standard check for comparing the performance of the newly developed genotypes. It is well known that individual genotypes are influenced a great deal by genotype × environment interaction and, therefore, a comparison of test genotypes with the yield of a check variety may not necessarily reflect true relative performance of the genotypes at all locations. To overcome the problem, Jensen (1976) suggested the use of two floating checks: (1) a preliminary check, the site highest yield (SHY) and (2) a secondary check, the site mean yield (SMY). Comparisons of individual genotypes in terms of the per cent of SHY or SMY are not influenced by the genotype × environment interaction product of a specific genotype included as a check in the tests. Site highest yield (SHY) is the maximum genotype × environment interaction product. It is the result of competitive environmental interaction experienced by the test genotypes against each sample of the common environment of the test location. A comparison of

genotypes in terms of the per cent of SHY ensures stability of performance, because SHY is a function of excellence which is not tied to a single genotype across sites as is the case with a check variety. The mean per cent SHY reflects the relative performance of each of the genotypes in relation with the environment of the different test sites. The positive characteristics are: (1) excellent stability, (2) goal-oriented (comparisons are related to the highest performance), (3) the "per cent SHY" index is always on a scale of 100 since SHY cannot be exceeded by any entry, and (4) the index shows not only rank but position of each entry.

Site mean yield (SMY) suffers from a lack of a clear-cut objective of selecting for high yield. The average performance of all genotypes across the test sites is not a very encouraging goal.

Jensen (1976) pointed out that in his cereal breeding programme test genotypes are rated in terms of per cent SHY to determine the yield potential of a genotype. The site mean yield (SMY), expressed as per cent of SHY provides a measure of average performance and an index for a cutoff point. The genotypes having lower per cent SHY than the SMY per cent of SHY are discarded. Standard variety checks continue to be included in nurseries but are used principally for public comparisons and to determine the improvement of new types over the existing standard types over the years.

Selection environment

An optimum environment for selecting the breeding material is one in which the difference between genotypes for the character under selection is maximised. In other words, the most appropriate environment is one in which heritability for the character under selection is maximum. As indicated earlier, breeding material is selected at two different stages that is (1) segregating early generations, and (2) advance homozygous lines. In the segregating generations, selection is made for highly heritable characters, such as disease resistance, while for characters of low heritability, such as yield, selection is delayed till later generations. Plant breeders are often faced with the question, whether emphasis should be laid on selection under different stress conditions in special nurseries (disease, drought, low fertility conditions, etc.) or under normal conditions of management and cultivation. Since development of special stress nurseries is expensive and requires considerable effort and labour to screen genotypes, a systematic approach to incorporate resistance goals should include an assessment of the cost and benefits of direct versus indirect selection. Yield evaluations under representative conditions of disease and insect infestation and environmental stresses that affect production in the region may be necessary.

If these factors are chronic problems, yield improvement may also improve the resistance of the crop. If, however, pest and environmental stresses are infrequent but acute and damaging, an evaluation of risk is appropriate before decisions are made on breeding for resistance, by screening under artificial conditions (Philip Busey, 1983). Generally, selection for resistance to stress environments will decrease both mean productivity across different environments and yield in non-stress environments. Therefore, a realistic assessment of the advantages of direct selection for a particular stress condition should be made in order to avoid the waste of effort on accumulating unnecessary resistance in the breeding lines. As a breeding strategy, it would be desirable to screen and evaluate the probable parental lines and the new germplasm for different stresses. While choosing the parents for hybridisation, due consideration should be given to resistance or tolerance of the lines. After hybridisation, selection and evaluation of the segregating lines under normal growing conditions may be more appropriate to retain a reasonable level of yield and required level of tolerance to the stress prevailing in a crop growing region.

Selection environment for yield has been a subject of considerable discussion in recent years. It has been argued that the varieties selected under high input management of experiment stations do not perform well under the low input and poor management conditions of the farmers' fields in the developing countries. Therefore, a suggestion is often made that selection for yield and productivity should be made under the stress conditions likely to be faced by a variety in the farmers' fields. However, several studies on the subject indicate that selection for yield and production traits under stress environment may not ensure the desired genetic gain.

Frey (1964) and Krull *et al.* (1966) observed that greater progress for yielding ability of small grains was expected if testing was done under high productivity and optimum management conditions. On the other hand, Gotoh and Osanai (1959) obtained highest yielding and widely adapted wheat lines from low fertility environment. Rosielle and Hamblin (1981) suggested that the choice of selection environment be made depending on the circumstances of the programme. If it is imperative that yields in stress environments be increased, then selection for tolerance may be worthwhile; but it should be recognised that this selection will generally decrease both mean productivity and yield in non-stress environments. If improvement in mean productivity is the objective, then direct selection for mean productivity should be done even though yields in stress environments may decrease. The most desirable approach would be to choose testing sites representative of production conditions for which a breeder wishes to improve mean yield. For example, if stress conditions occur randomly in one out of three seasons in a breeding region, then one of three testing sites should be a stress

environment and selection should be practiced for mean yield. In early generations, the segregating lines should be evaluated under good management conditions in order to identify good productive lines since genetic variance is maximised under the non-stress environments (Frey, 1964; Gotoh and Osanai 1959; Johnson and Frey, 1967, and Mederski and Jeffers, 1973).

Population improvement and recurrent selection

In recent years there has been a growing feeling that the conventional breeding programmes of the self-pollinated crops suffer from narrow genetic base and limited genetic recombination. The use of three-way, double and composite cross populations broadens the genetic base of the segregating population to some extent. But due to selfing, the conservative forces of linkage restrict reshuffling between the genes coming from diverse parents to family lines of descent. A number of workers have suggested the use of genetic male sterility and treatment with mutagens to increase out-crossing. Random mating between selected genotypes by controlled hand pollinations, for increasing recombinations in heterogeneous base populations has also been suggested.

The success of population improvement programmes depends on the accuracy with which genetically superior genotypes can be identified and inter-mating done among them (Humphrey *et al.*, 1969). Just random intercrossing on limited scale in self-pollinated crops is not likely to increase the population mean.

The various systems of intermating to foster recombination, and selection of superior gene combinations, in self-pollinated crops are still in the stage of infancy and results of conspicuous applied value have yet to come. Some breeders and geneticists have expressed scepticism about the advantages of a high degree of random mating and reshuffling of the genes, which have reached a genetic balance as co-adapted gene blocks in self-pollinated crops (Pederson 1974; Boss, 1977). However, Miller and Rawlings (1967); Baker (1968); Matzinger and Wernsman (1968); Meredith and Bridge (1971); and Yunus and Paroda (1982) reported increased genetic variance from random intermating and possibility of obtaining useful recombinants as compared to selection without intermating. In contrast, Altman and Busch (1984) working with wheat, concluded that random intermating within single cross populations resulted in insufficient useful recombination to justify its use as a primary breeding procedure prior to selection. The approach of population improvement and recurrent selection in self-pollinated crops requires thorough investigation and development of procedural details, particularly related to the selection phase of the programme.

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3

Concepts and Methods: Cross-pollinated Crops

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Cross-pollinated species, or outbreeders, are highly heterozygous, heterogeneous and carry a higher mutation load than the inbreeders. Their mode of reproduction bestows upon them considerable heterozygous balance under panmitic population structure. Inbreeding such species disturbs the natural rhythm and leads to inbreeding depression. It is, therefore, essential that if selfing has created any disturbance in outbreeders, the heterozygous structure be restored. Since artificial selfing and outcrossing can be carried out in such crops at will, all breeding methodologies, even those meant for self-pollinated crops, can be used for their improvement. In fact, allogamous crops have provided opportunities for innovating breeding procedures to achieve the maximum efficiency by combining selfing and outcrossing cycles.

Thus, the classical separation of breeding procedures for self- and cross-pollinated crops has become unnecessary, particularly, for outbreeders which are amenable to selfing. Exploitation of heterosis has been the major objective in cross-pollinated crops and most breeding procedures developed earlier aimed at maximising heterosis. In later years, with better understanding of genetic structure of populations and availability of efficient biometrical methods of estimating genetic parameters, newer procedures of accumulating desirable genes have been developed. The presently available breeding procedures for cross-pollinated crops can be broadly classified under two heads: (1) Hybrid development, and (2) Population improvement. Both these methodologies, though independent, are not mutually exclusive. The steps for operating the two procedures, however, have contrasting requirements. For hybrid development, fixation of genes through cycles of selfing is necessary whereas such fixation is avoided while developing populations. As can be seen from the scheme given below, in both cases the homozygosity attained through initial selfing is converted to heterozygosity at the end of breeding programme. Irrespective of whether hybrids, synthetics or composites are being developed, great emphasis is placed on the attainment of homogeneity in the end products.

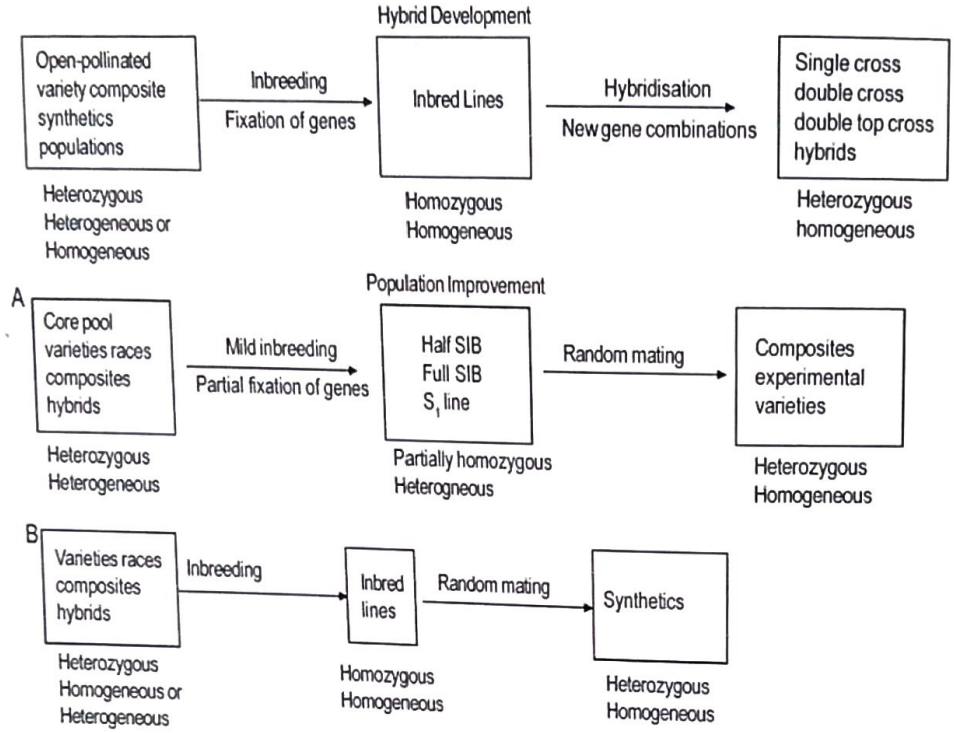

Fig. 1.

All breeding methods must identify, select, and develop the most promising recombinant or progeny. For doing this, a number of selection procedures have been devised. Some procedures are applicable to both hybrid development and population improvement. These procedures will be described under the respective breeding methods.

Hybrid Development

Hybrids are first generation (F_1) crosses between genetically unrelated parents which may be pure lines, inbreds, varieties or populations. Most of the commercial hybrids of cross-pollinated species have originated from two or more pure lines (inbreds). This is because when attempts were made to develop varietal hybrids using heterozygous varieties of maize as parents they could not succeed commercially because of low hybrid vigour.

Since the pioneering work of early maize breeders during the period 1908 to 1946, which earned them the acronym 'hybrid corn makers', (Richey, Jenkins, Hayes, Shull, Jones, and East) very little has changed in procedures employed for hybrid maize breeding. The procedures developed for maize are equally applicable for producing hybrids in other cross-pollinated crops. To be successful, hybrid approach must fulfil the following conditions:

1) Existence substantial non-additive component of gene effects namely, dominance, overdominance or epistasis.

2) Availability of genetic diversity.
3) Availability of cytoplasmic male sterility greatly aids production of hybrid seed economically for commercial success.

The standard hybrid breeding involves the following three basic phases:

A) Development of inbred lines;
B) Testing of inbred lines;
C) Combination of inbred lines into heterotic hybrids.

Development of inbred lines

Source or base population

In cross-pollinated crops, inbred lines are produced by selfing individual plants. The extraction source of inbreds can be open-pollinated varieties, composites, synthetics, germplasm complexes or any other heterozygous population. The choice of source or base population for inbred derivation is of extreme importance for the consequent performance of hybrids. To obtain maximum advantage for selecting elite inbred lines, the base population must be genetically broad based and be of high *per se* performance that is a reservoir of most desirable genes. In fact, restricted variability of base population has been the major genetic limitation for exploitation of hybrid approach. Narrow gene pools result in inbreds and consequently hybrids, of low yield potential. To elevate the yield potential of inbred lines, it is essential to improve the source population. Recent population improvement procedures have provided the crucial methodology of increasing the level of performance of base populations so that promising inbred lines can be isolated. In fact, the commercial success of single cross hybrids of maize in recent years can be attributed to this methodology. This aspect will be dealt with in detail later.

Methods of inbred derivation

Standard method: Selfing is carried out on selected plants in the base population. Selection of plants to be selfed is usually visual. Major visual selection criteria are vigour, stalk strength, height, maturity and pest and disease resistance. It should be remembered that the selection of vigorous plants during inbreeding neither delays attainment of homozygosity nor does it affect combining ability. At harvest, ear characters like size, grain type, grain colour and freedom from diseases are considered. The selected ears or heads are planted ear-to-row in the following season and selection is exercised both within and between progenies. Only the most desirable plants within superior progenies are carried to the next inbreeding cycle. Three to four generations of self-fertilisation and selection resolve the population into distinct true breeding types.

Single hill method: This method is a modification of the standard method of Jones and Singleton (1934). Here, the usual progeny row is represented by a single three-plant hill in each cycle of inbreeding. The procedure allows testing

of a larger number of progenies in the same space. This method, however, reduces the scope for within progeny selection while improving between progeny selection. In the initial cycles of inbreeding, therefore, this method is useful because it helps in discarding a large number of poor progenies.

Homozygous diploids: This method of inbred derivation has been tried only in maize so far. Use of doubled haploids to produce true breeding homozygous diploid lines was suggested as early as 1943 (Einset 1943). Chase (1952a) reported spontaneous occurrence of androgenic haploids with a frequency of 1 per 100. Among the haploids, selfed progenies could be obtained with a frequency of about 1 in 10. Thus in nature, it was possible to obtain one homozygous diploid in 10,000 plants. This frequency was too small to be useful for breeding purposes. Lately, however, it has been possible to increase the frequency of haploids up to 5 per cent by using inducer stocks (Sarkar and Coe, 1978). The use of suitable markers (such as the scutellum marker in maize) for detecting haploid seeds and inducer stocks to increase their frequency, homozygous diploids from doubled haploids are now gaining popularity as the quickest method of inbred derivation. Seed companies, such as, Dekalb in the U.S.A. have used this method to isolate inbreds for a number of hybrids of corn.

Three other methods, that is, pedigree selection (Hayes and Johnson 1939), gamete selection (Stadler, 1944) and early testing (Jenkins, 1935; Sprague, 1946) have also been used for inbred development. These methods can be used for deriving superior inbred lines or for improving the already developed inbred lines.

Methods of obtaining superior inbred

In the standard method of producing inbred lines, inbreeding is carried out in an open-pollinated variety and the selected lines are utilised for the production of hybrids. This procedure yields superior inbreds with low frequency. Methods that will improve this frequency or will allow improvement of the existing inbreds can greatly contribute to the success of hybrid variety programmes. In the following some of the methods developed for meeting the objectives are described:

Pedigree method: This method differs from the standard method only in the make-up of the original parental population. In the pedigree method, the starting material is a single or a double cross hybrid. It is assumed that inbreds derived from high yielding hybrids will have elevated and superior performance for disease resistance and yield and will, in turn, produce promising hybrids. Results obtained in maize, however, do not wholly substantiate these expectations.

Cumulative selection: This method, given by Richey (1945), advocates visual selection for desirable characters to develop inbred lines by the standard method. Every cycle of inbreeding subdivides the original population into different families. These families are selected on the basis of top-cross test (either for gca or

sca). The selected families are then crossed and further inbreeding is carried out in the superior crosses. This method accumulates desirable genes and increases the chances of obtaining superior inbred lines. It has also been observed that inbred lines derived from crosses of high combining parents tend to be high combiners.

Gamete selection: This method is based on the premise that "in a variety in which one per cent of the open-pollinated plants attain a desired level of yield potential, gametes of correspondingly high level must constitute almost 10 per cent of the gametic population". First suggested by Stadler (1944) this method aims at replacing an inferior inbred line in a hybrid by a superior counterpart. The unit of selection is a gamete and the material on which selection is operated comprises composites, synthetics, hybrids or open-pollinated varieties. The inbred to be improved is crossed with the source. The F_1 plants are simultaneously selfed and crossed with a tester; the choice of tester depending on the objective. The tester may be a hybrid or a composite. The original inbred is also crossed with the tester to provide a check in the test-cross trial. Any test-cross outyielding this check is presumed to have received a better gamete. The superior gametes are recovered as F_2 selfs. Selection and selfing is continued till desirable uniformity is attained.

Convergent improvement: This method, suggested by Richey (1927), is a plan for improving inbred lines without altering their proven combining ability. It utilises double backcrossing to improve the characters of the two parental inbreds. If the lines are A and B, the scheme involves backcrossing F_1 (A × B) to A and selecting for desirable traits of B. Simultaneously, (A × B) is backcrossed with B and the selection for desirable traits of A is made. After about three backcrosses, combining ability is largely recovered and selfing is done to fix the selected genes. This method is useful for improving such characters as vigour, resistance to diseases and pests and lodging resistance.

Recurrent selection: Recurrent selection procedures represent a special set of breeding techniques aimed at increasing the proportion of superior genotypes in the heterozygous source material without allowing rapid fixation. Such sources may be used as base material for deriving superior inbred lines which are called 'second cycle' or 'third cycle' inbred lines. The source may also be early generation inbreds with high *per se* performance and high combining ability. Since this procedure improves the genetic constitution of heterozygous population without rapid fixation, it represents a genetic bridge between the hybrid and population improvement approaches. The subject will be further discussed under population improvement.

Testing of Inbred Lines

Testing or evaluation of inbred lines poses a problem more complex than derivation of inbred lines. The problem has both theoretical and practical aspects. It is customary to derive thousands of S_1 progenies from different source

populations but only a fraction of these can be carried forward to S_2 and later cycles of selfing. It is, therefore, essential that during the initial stages of inbreeding, selection is exercised to reduce the number of inbred lines to a manageable limit. Visual selection based on vigour is used as the first discriminating step for selecting plants for further inbreeding because it has been found that an inbred derived from a vigorous plant is more likely to yield a good combiner. Since visual parameters are not definitive criteria for selecting combining ability, the final test has to be the performance of hybrid combinations. Initially, all possible crosses are made and their performance is evaluated in trials. Since $n(n-1)/2$single crosses can be made out of n inbreds, it becomes impossible to make and test so many single crosses in a breeding programme where a large number of inbreds have to be evolved. A simpler method of evaluating inbred lines, known as 'top-cross test', was, therefore, devised. This method allows not only testing of a large number of inbred lines at one time but also provides basis for discarding more than 50 per cent of the tested lines.

Time of testing

Opinions still differ considerably regarding the best time or stage for evaluating inbred lines. The usual procedure has been to evaluate the inbred lines in top-crosses after they have attained a high degree of homozygosity that is the inbred lines have been produced by three to four generations of selfing. However, it was suggested that the process of inbreeding for deriving inbreds and their top-cross evaluation could be carried out simultaneously. This method, known as 'Early Testing', saves considerable time (Lonnquist 1950; Sprague, 1946, 1952). Jenkins (1935), who suggested the early testing procedure, assumed marked differences in: (a) combining ability among the plants of a population selected for inbreeding, and (b) the time taken for the establishment and expression of combining ability during inbreeding. Sprague (1946) and Lonnquist (1950) have provided experimental data supporting the validity of early testing for detecting lines with high combining ability. Wellhausen (1952) has shown that early identification of S_1 lines with superior combining ability plays an important role in the success of a breeding programme. An important objection against early testing is that the performance of inbred lines in crosses may change with the degree of homozygosity attained by the lines. Early generation testing for combining ability is not desirable when high combining lines are agronomically poor and are likely to be eliminated during inbreeding. However, where the material has previously been selected visually for desirable characteristics like vigour and stalk strength early generation testing is profitable for isolating families of high combining ability.

Top cross testing

Since it is now agreed that the best criterion of judging the worth of an inbred line is its performance in hybrid combinations, top-cross testing is regarded as the easiest and quickest method of eliminating the bulk of poorly combining inbred lines. Top-cross is based on the general combining ability which measures the ability of an inbred line to produce superior hybrids in combination with other inbreds. As stated earlier, however, top-cross testing should be done only on inbred lines which are acceptable on agronomic standards. After all, an inbred poor in vigour or susceptible to diseases, is of no use even if it performs excellent in hybrid combination since the inbred parent itself cannot be maintained and multiplied easily.

Top-cross (also known as inbred × variety cross) involves crossing of a large number of inbreds with a common heterozygous and heterogenous tester possessing wide genetic base. Selection based on top-cross performance identifies the best general combiners.

Combination of inbred lines into heterotic hybrids

We have seen that top-cross provides the means of identifying promising inbred lines from a group of size n with only n crosses instead of $\frac{n(n-1)}{2}$ crosses. After the promising inbreds have been identified on the basis of general combining ability in the top-cross, it is necessary to know the specific single, three-way or double cross combinations that will give the highest yield. Since the high yield of a hybrid is the expression of heterosis, consideration of the gene action involved assumes importance. The gene action can be estimated from specific combining ability (sca) and includes non-additive gene effects like dominance and epistasis. Selection of outstanding single crosses, based on sca, is done from the single cross performance in a trial. Usually, a diallel mating system involving all possible combinations among the selected inbred lines is adopted. The number of single and double cross combinations increases rapidly with the increase in the number of inbred lines. For instance 20 inbreds can be combined to produce.

$$\frac{n(n-1)}{2} = \frac{20 \times 19}{2} = 190 \text{ single crosses}$$

and

$$\frac{3n!}{4!(n-4)!} = \frac{3 \times 20!}{4!\ 16!} = 14{,}535 \text{ double crosses}$$

It is obvious that breeding so many double crosses is impossible. Some method of predicting the double cross performance, without actually making the crosses, is necessary so that only the most promising crosses are made and evaluated. Commercially acceptable single cross hybrids (hybrids whose inbred parents are also high yielding), however, can be identified on the basis of yield trial of all possible single crosses.

Prediction of double cross performance

Prediction of double cross performance from single cross data has been a routine practice adopted by maize breeders. The first studies on this aspect were reported by Jenkins (1934) who gave the following four methods of prediction:

a) The mean performance of the six possible single crosses among any set of four inbred lines represents the predicted double cross performance. For example, considering inbreds A, B, C and D,

$$= \frac{AB + AC + AD + BC + BD + CD}{6}$$ Predicted performance of double cross ABCD

b) The mean performance of the four non-parental single crosses represents the predicted double cross performance. According to this method,

$$\text{Predicted performance of double cross ABCD} = \frac{AC + AD + BC + BD}{4}$$

c) The mean performance of a set of four lines over a series of single crosses that is performance of A, B, C and D separately in single cross combination with other inbreds E, F, G, H ... etc. predicts the double cross performance.

$$\text{Thus, predicted performance of ABCD} = \frac{AE + AF + AG + AA + BE + BF + BH + CE, CE + ... + DH}{n}$$

where n = number of single crosses

d) The mean performance of the top-crosses of the four inbred lines involved in the double cross represents the double cross performance for example,

$$\text{Predicted performance of double cross ABCD} = \frac{(A \times \text{variety}) + (B \times \text{variety}) + (C \times \text{variety}) + (D \times \text{variety})}{4}$$

Of these four methods, method (b) has been found to give the most accurate estimate of the double cross performance.

Development of multiple hybrids and synthetics

So far we have considered the use of elite inbred lines to produce either single cross hybrids or double cross hybrids. Another use to which inbred lines have been put is in the development of multiple hybrids and synthetics.

Multiple hybrids represent the combination of a larger number of inbred lines in the F_1 generation (A × B × C × D × E × F × G × H) F_1 where A, B, C, D... H are inbreds lines of high gca. Multiple hybrids have not been extensively studied but it has been found that they do not outyield the double cross hybrids. Moreover the difficulty of identifying and isolating a large number of inbred lines which possess high general combining ability *inter se* has restricted the commercial production of such hybrids.

Synthetics or synthetic varieties are advanced generation of multiple hybrid increased by random mating in isolation. Hayes and Garber (1919) were the first to suggest the possibility of commercial production of synthetics. Synthetics produced through recombination among several selfed lines have one advantage over hybrids—the farmer can save his requirement of seeds from his own crop. In cross-pollinated crops, where pollination control is not easy (for example cocoa, alfalfa and clovers), synthetics are of great value.

A synthetic variety is synthesised from inbred lines which have been tested and found to show high gca. This is done to ensure that the inbreds involved combine well when crossed *inter se* giving high yield in F_1 and less depression in the advanced generations. The possible advantages of synthetics are;

1) In fringe areas where cost of hybrid seed is very high or availability is poor, farmers can keep their own seeds.
2) Greater variability in synthetics permits more flexibility against changing environmental conditions, diseases and pest infestations.
3) Synthetics may have a place where commercial acreage is too small to support the hybrid seed industry.

In addition, synthetics are valuable reservoirs for desirable genes. Synthetics derived from early generation inbred lines have given encouraging performance in maize. Steps for the derivation of synthetics from early generation inbred lines are:

a) Isolation of one generation selfed lines (S_1).
b) Testing these lines in top-crosses for high gca for yield and other characters.
c) Intercrossing the superior lines to produce the F_1 generation of synthetic.
d) Advancing the F_1 synthetic in isolation under open pollination.

Population Improvement

Sprague (1966) used population improvement as a general term to include all operations within a system designed to yield a sexually propagated improved type. The system may be a random mating population or pure line. The term, as applied to cross-pollinated species, refers to cyclical improvement in the mean performance of a random mating population of genotypes which have undergone, or are undergoing selection but in which the final product retains a high degree of heterozygosity. Genetic concept: Population improvement is based almost entirely on the concept of utilising additive gene effects. A continuous accumulation of additive genetic effects leading to gradual improvement is sought without reaching a dead end. Population improvement may take many forms. In each form the selection procedures determine the efficiency with which additive genes are exploited without leading to complete fixation. The additive genes can be exploited in two ways since it is recognised that the manifestation of heterosis need not depend entirely on complete dominance (as was the earlier thinking). Partial dominance at many loci can also evoke heterosis. The additive effects arising out of this type of gene action are easily manipulated by simple selection schemes and lead to improved population performance. Such populations or their derivatives provide the second avenue of exploiting additive effects. Material from such populations can be used as parents in a hybrid programme for extracting superior inbreds or as sources for better composites. The various genetic concepts relevant to population improvement may be summarised as follows:

Presence of considerable genetic variance for the different traits of importance.

1. Presence of high additive genetic variance with partial dominance in the source population.
2. Presence of high heritability in the traits. This follows from (2) above as heritability is a function of additive genetic variation in relation to total genetic variation.
3. Absence of undesirable linkages between characters for which improvement is sought.
4. Absence of negative association between desirable traits. For instance, selection for early maturity leads to decrease in yield.
5. Availability of considerable variability for diverse genes controlling desirable traits which have not undergone any previous selection. Such genes provide greater scope for creating a wide genetic base in the initial population.

A base population which fulfils the above requirements is expected to respond to improvement by any method. The choice of a procedure of population improvement applicable to any base population would, however, depend on several other criteria, such as:

Operational simplicity

In general, population improvement schemes are simple and demand much less technical knowledge and resources (for example physical facilities, etc.) in comparison with hybrid breeding. The different procedures possess a varying degree of operational simplicity. For instance, mass selection is the simplest to operate and involves no artificial pollination or special evaluation techniques. Ear-to-row or half-sib procedures also utilise natural open-pollination. In other procedures such as full-sib selection and recurrent selection, however, artificial hand pollination becomes necessary.

Number of generations needed to complete one cycle of selection

Different population improvement schemes require different number of generations for the completion of one cycle. For instance, mass selection or ear-to-row selection require one to two generations for completing one cycle, whereas recurrent selection procedures require four generations per cycle for completion. Under such situations, the choice of a scheme requiring more number of generations per cycle will slow down the progress of improvement.

Gains from selection

It is most important consideration in the choice of selection procedure. Genetic gain can be predicted from the following formula (Eberhart, 1970):

$$\Delta G = kc\ \sigma^2 g/(y\sigma p)$$

Where k is selection differential, c is a function of the parental control, $\sigma^2 g$ is the additive genetic variance among progenies, y is the number of years required per cycle and σp is the phenotypic standard deviation among progenies. It is evident that genetic gain can be increased by increasing parental control or reducing number or years per cycle or improving the precision of the evaluation of progenies. Breeding and selection procedures favouring increase in additive genetic variance tend to improve the genetic gain per cycle.

Depletion of variability

Selection procedures differ in their capacity to release or fix the variability present in the original population. The choice of procedure, therefore, involves the important consideration of whether larger immediate gains with greater fixation of types is desired or whether there should be a compromise between the two, so that, residual variability in the population is retained to permit scope for further improvement. Both the alternatives are influenced by selection differential and selection intensity of the programme. Residual variability in population improvement programme should be of the additive genetic type so that it contributes to gains from selection. This is a realisable objective because

Gardner (1969) reported the presence of substantial additive effects even after 14 cycles of mass selection. Similarly, Hallauer (1970) reported residual additive effects of considerable magnitude after four cycles of reciprocal recurrent selection.

Possibility of simultaneous improvement in many characters

The population improvement procedure should provide ample scope for the improvement of various agronomic traits simultaneously. Also selection for improvement of one character should not lead to impairment in other desired attributes or concomitantly introduce undesirable characteristics. In maize, for instance, mass selection for yield has been found to result in late maturing and tall populations.

Objective of the improvement programme

The ultimate visualised use of improved population will also determine the choice of the scheme deployed. If derivation of superior inbred lines for new high yielding hybrids is the goal, recurrent selection procedures (for general combining ability) will be the logical choice. On the other hand, if replacement of one inbred in a hybrid is the objective, then recurrent selection for specific combining ability should be preferred. Use of half-sib and fullsib procedures are good overall, both for getting high yielding composites or serving as a better source for the derivation of new inbred lines.

Population improvement procedures

Population improvement procedures are designed to increase the frequency of desirable genes in the population. As has been stated earlier population improvement depends on the exploitation of additive component of genetic variation. The various schemes of this exploitation differ only in their ability to capitalise on different fraction of the additive genetic variance. In recent years many breeders (Gardner, 1978; Hallauer and Miranda 1981) have classified the population improvement approach into two major categories:

i) Intra-population improvement.

ii) Inter-population improvement.

The basic difference in the two lies in the nature of variability in the initial population on which the breeding procedures are applied. In the intra-population improvement techniques are devised to manipulate the intrinsic variability existing in a population. In the latter, techniques are devised for creating new variability and to simultaneously generate heterosis expression in the population by making crosses with genetically diverse population at some stage. It is, however, obvious that selection procedures, which may or may not be common to the two procedures, represent the most important aspect for recognising and isolating desirable gene combinations for cyclical improvement. Most selection procedures,

except mass selection, are applicable to both the above types of population improvement. In recent years, several modifications of the breeding procedures involved in population improvement have been suggested but all relate to the number of plants sampled as males or females, mode of selection of progenies for recycling or ways of bringing about the crossing and subsequent recycling. All population improvement procedures can be broadly grouped under recurrent selection since, in general, the methods involve three operations: (1) development of progenies, (2) evaluation and selection of progenies, and (3) recombination of superior progenies to generate the next cycle of population. Since the recurrent selection procedures differ depending on the crop they are applied to and the amenability of the crop to selfing and crossing, these will be dealt with separately. In the following, the basic population improvement procedures are discussed.

Mass selection

This is the oldest method of selection for cross pollinated crops. In its simplest form, it consists of selection of desirable plants and bulking their seeds for planting in the following season. It is phenotypic *per se* selection without any information on the progeny performance. The efficiency of this selection procedure is limited by lack of control on male parentage, lack of information on progeny performance and the confusing effect of soil variability on the phenotype of the plants.

Considerable controversy exists regarding the efficiency of mass selection for improvement.

Modified mass selection

The question of effectiveness of mass selection was reexamined by Gardner (1961) and several procedural modifications were suggested. These included: (a) the selected population is grown in isolation to minimise introgression from other stock; (b) a grid or sub-plot system of harvesting is used to minimise effects from environmental and soil variability; and (c) adequate amount of remnant seed is retained to permit subsequent evaluation of progress.

In the modified mass selection, the population is grown in isolation at a low plant density to allow better expression of individual phenotypic differences. The whole area is divided into sub-plots or grids. At harvest, ears or heads are selected within each grid. From the seed of the selected ears, three sets are made; one set is used for planting in the next season, another set is used for testing the performance and estimating gains and the third set is kept as reserve seed. Modified mass selection is also known as stratified mass selection.

As mass selection is largely based on *per se* performance, its success depends not only on the presence of high additive component, which means lower G × E interactions, but also on the presence of high genotypic variation. In other words,

mass selection is more successful in a highly heterogeneous and heterozygous population. Also, since mass selection does not take progeny performance into account, high heritability in the trait under consideration will give better results.

Ear-to-row selection

This method involves selection of a number of phenotypically desirable ears out of a population grown in isolation. The seeds of the harvested ears are not bulked (as is done in mass selection) but are kept separate. A part of the seeds of each ear is grown in the next season to obtain information on progeny performance. Based on this progeny performance, the next cycle is taken from the remnant seeds of the promising ears. This method was applied as early as in 1899 by Hopkins to improve traits like oil and protein percentage of the corn kernels. Though this method became very popular with early maize breeders, its effectiveness was found to be poor for characters of low heritability, particularly for yield. The lack of response for yield can be attributed to various causes such as:

a) Inefficient and inadequate field plot technique which fails to distinguish between progeny performance from single row plots (seed number being limited).

b) Continued ear-to-row selection results in inbreeding and hence loss in vigour.

c) Lack of consistency in performance from one generation to the other. This may be a reflection of the fact that the selections were probably hybrids of unknown parentage.

d) Lack of high heritability for the trait concerned.

Modified ear-to-row selection

Several modifications have also been suggested over the original ear-to-row procedure to overcome specific difficulties. Lonnquist (1964) proposed a modified 'ear-to-row' selection scheme which evaluates progenies in several locations and shortens each cycle to one season. The steps of this modified scheme are:

1) A large number of ears (100 or more) are selected from a variety, a composite, a synthetic, a population, germplasm or a gene pool. The selection is purely on the basis of grain and agronomic characters of the plants. At harvest, each ear is shelled separately.

2) From the seeds of each selected ear, progeny rows are grown in a single replication at different locations representing the range of environment in which the future variety is to be grown. Each replicate also includes the best check and the original base population for comparison.

3) The replicate at the main station is handled as a crossing block. A balanced mixture is used as the pollen parent. The balanced mixture is a composite of equal number of seeds from each progeny row (that is, equal number of seeds are taken from each progeny and mixed together). This compositing permits the maximum opportunity for each progeny row to cross with all other progeny rows.
4) At the main location, five phenotypically best appearing plants in each of the progeny are marked. When each row is harvested and weighed, the five marked ears are placed in a bag.
5) On the basis of an average performance overall locations, only the top 20 per cent of the progenies are selected. The five ears from the selected progenies (in these 20 per cent of the progenies) are sown in the next season in ear-to-row in the manner described earlier.

Half-sib progeny selection

Half-sibs are a family of individuals having one parent common namely, individuals having either the same father or the same mother. There are several schemes of half-sib breeding. Sprague (1966) has differentiated between half-sib progeny test and half-sib test on the basis of how the new population has been formed.

Under the half-sib family scheme, plants used as males are selfed and also crossed to several females to produce half-sib families. The selected plants are composited from the selfed seeds. In half-sib test no selfing is carried out and compositing is done from open-pollinated seeds. It may be remembered that top-cross test (as used in hybrid development), ear-to-row selection and recurrent selection procedures (with the exception of simple recurrent selection) can be considered as variants of half-sib breeding. The North Carolina Mating Designs I and III also utilise half-sib mating system.

In recent years, half-sib-approach in association with ear-to-row selection has been used in a big way for population improvement in maize. The maize breeding programmes of India and CIMMYT have used this modified procedure as a routine initial improvement methodology.

The procedure involves the formation of a wide and genetically diverse gene pool using various kinds of germplasm, composites, synthetics, hybrids, varieties and races of both indigenous and exotic origin. Lonnquist (1967b) outlining the requirement of such a gene pool for the initiation of population improvement programme in maize, states: "Formation of a germplasm pool should be the compositing of available material collections reasonably well adapted to the area for which improved populations are sought. If endosperm types are an important consideration, only those varieties embodying desired types would be used. The compositing of a number of varieties would result in increasing the genetic variability and assure greater ultimate selection potential".

After the development of a gene pool, half-sib method of selection (ear-to-row) is applied to obtain improvement. This stage has been termed as "Back Up Stage" and the pools undergoing improvement by half-sib selection are called "Core Pools". The details of the procedure are outlined below:

1) For the development of core pool, a large number of germplasm (more the better) having similar maturity range are selected. About 30 to 40 genotypes, desirable from the agronomic point of view, are physically mixed in equal amount. These are grown in an isolated recombination block (usually one to two acres). Before flowering, agronomically undesirable plants and plants susceptible to insect/disease are removed. At the time of harvest also, a mild selection for plant height and ear size is exercised. About 600 to 800 ears are selected. .

For the second cycle of recombination, equal quantities of seeds of selected ears are again mixed and grown in isolation block as before and the process is repeated. The number of cycles of recombination needed will, of course, depend upon the initial genetic diversity among the constituents. The breeder is the best judge of determining this number. Usually, two to four cycles are sufficient for a good mixing. The products of recombination cycles represent the "Core Back Up Pool" and is usually formed of 250 half-sib ears kept separate at the time of harvest.

The back up stage has a dual role of development of core pool to maintain continuity of improvement and to achieve further improvement. After each cycle, the selected families, are put into recombination block along with new donors to improve the original core pool. The unproven donors must be used as females and detasseled, so as not to contaminate the original pool.

2) These 250 half-sib families undergo testing in the main season. Replicated progeny tests, along with the best checks, are conducted at more than two locations. In addition to yield test, one replication each is grown for disease resistance screening (disease nursery), insect resistance screening (insect nursery) and population stress screening (high density nursery). As far as possible, artificial infestation and inoculation techniques are used in the disease and insect nurseries.

Based on the data from yield trials and the three screening nurseries, elite families are selected. In the initial stage, it is important that mild selection pressure is applied. Usually 125 to 175 families are selected to regenerate the next cycle of half-sib families. As more and more cycles are taken, selection pressure is increased gradually and at a later stage, 70 to 80 families may be sufficient to derive the next cycle.

The regeneration of successive cycles of half-sib progenies is done from the selected progenies grown from the remnant seeds in a half-sib nursery in isolation. The male rows represent the balanced mixture of seeds of the selected families

(that is, an equal amount of seeds taken from all the selected families are mixed and grown as male rows). All the selected progenies are grown as female rows. At harvest, the ears from each family are selected on phenotypic basis or on the basis of mean performance of the whole progeny row. Usually 250 ears are kept separate for half-sib progeny testing.

From the elite selected families, the top superior families (say 10 to 20, depending upon the uniformity within and between families) are grown in the next season from the remnant seeds. Random mating in isolation of selected superior families is done to obtain the experimental variety. The selected experimental varieties are tested for their performance in multilocation trials.

Full-sib progeny selection

Full-sibs are a family of individuals having both parents common and are derived from selfing or crossing of two plants from the same population. Full-sib utilisation provides for greater control of additive effects. This method has not been widely used in cross-pollinated crops. However, in maize, Hallauer and Eberhart (1970) suggested use of prolific plants for producing full-sib progenies by making a reciprocal cross between two plants while at the same time selfing the other ear. Remnant seeds of the selfed ears of the selected full-sib progenies are used to derive the next cycle.

A modified and more practical, approach to this method has been used at CIMMYT and in the Indian maize programme in recent years. The various steps of the method are as follows:

1) The base population in which full-sib progenies are to derive consists of, either, back-up populations (as described in half-sib method) that have undergone a few cycles of half-sib selection, or, any population that has been synthesised using desirable donors. Full-sibs are developed by making crosses (sibbing) between phenotypically desirable and vigorous plants within the population.

2) The best 250 ears (or less if so many good ears are not available) are selected. Each ear represents a family of full-sibs. A part of seeds from each ear is planted in replicated yield trials (two replications) disease nursery (one replication), insect nursery (one replication) and if need be high density nursery (one replication). Disease and pest screening is done under artificial inoculation and infestation. Families are selected on the basis of performance for yield, disease and pest resistance and high density stress.

3) Selected families (80 to 100) are planted ear-to-row in the breeding nursery from the remnant seeds. Crosses are made between the best plants of selected families, taking care that repeat or duplicate crosses are not made between the same two families. These crosses form a new set of full-sib progenies and their number is kept at 250 again for uniformity and convenience.

4) The cycle is repeated again. At the completion of the requisite number of cycles of selection, indicated by decline in perceptible gains, 10 to 15 top phenotypically similar progenies may be recombined in isolation to generate experimental variety (composite) for testing and commercial release.

Integrated population improvement and hybrid development approach

In the early seventies, the stagnating yield levels of the hybrids and the quest for better performing inbred lines prompted the plant breeders, particularly maize breeders, to integrate population improvement procedures with hybrid development. Statistical genetic theory indicates that it is possible to obtain improved breeding populations through inter-population improved system. The hybrids derived from such populations show a corresponding improvement in performance (Fig. 2).

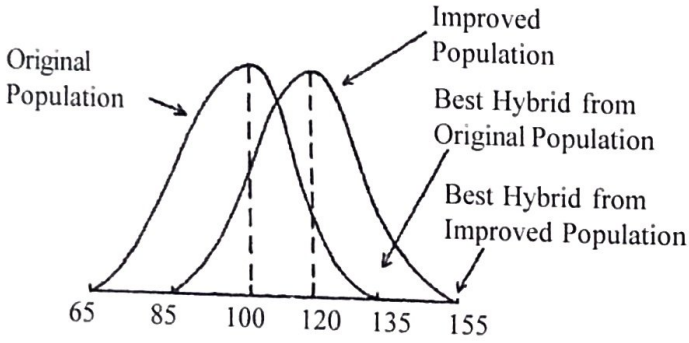

Fig. 2. Expected improvement of hybrids derived from improved population (Sprague and Eberhart, 1977)

It is, therefore, possible to derive progressively better performing inbred lines after each cycle of population improvement. The procedure of inbreeding and hybridisation operated in each cycle can be used to develop superior hybrids. Full-sib reciprocal recurrent selection has also been suggested by several workers (Hallauer, 1973; Lonnquist and Williams, 1967) because of the expected exploitation of all types of gene action while developing single cross hybrids. Hoegemeyer (1974) showed that hybrids developed from selected reciprocal pairs of inbred lines give superior yields because of specific combining ability. These lines also have good general combining ability.

The full-sib progenies from the advanced cycle of intra- or inter-population phases can serve as parents of single crosses. The hybrids so developed would be superior to the ones derived by traditional method (Mukherjee and Ahuja 1986).

Recurrent selection procedures

Hull (1952) defined recurrent selection as "reselection, generation after generation, with interbreeding of selects to provide for genetic recombination".

Thus selection among isolates is not recurrent selection; it becomes recurrent only when selects are interbred and a new cycle of selection is initiated.

Recurrent selection procedures were developed for extracting higher yielding genotypes from an improved base population. The proportion of superior genotypes recurring in a heterozygous population is a function of the frequency of desirable genes in the gene pool. The higher the frequency of desirable gene combinations, greater will be the probability of getting better genotypes. Recurrent selection procedures, in fact, represent a sort of population improvement. The difference is that they allow mild inbreeding and tend to increase the frequency of desirable genes, thus increasing the efficiency of selection. The basic steps in the recurrent selection, outlined by Jenkins (1940), are:

1) Isolation of one-generation selfed lines.
2) Testing of these lines for yield and other characters.
3) Intercrossing the best selfed lines to produce new improved population.

It is thus clear that heterozygosity lost during selfing is recovered at the end in the form of desired gene combinations. Four types of recurrent selection are known:

a) Simple recurrent selection.
b) Recurrent selection for general combining ability.
c) Recurrent selection for specific combining ability.
d) Reciprocal recurrent selection.

a) *Simple recurrent selection*

A number of phenotypically desirable plants are selected and self-pollinated. At harvest, selection is again done for desirable plant and ear characters. Progenies from these are grown in the next season and all possible crosses are made. At harvest, these crosses (equal amount of seed from each cross) are bulked for the next cycle of recurrent selection. Simple recurrent selection requires only two seasons for the completion of one cycle. Actually, this method is an extension of mass selection. The difference between simple recurrent and mass selection are first selfed and are then manually crossed before another round of selection. This method is useful only where the characters have high heritability, because it is operated on phenotypic basis.

In the simple recurrent selection, one cycle can also be completed in only one season when the desired selections can be identified before flowering. In such a situation intercrosses between selections can be made in the same season so that growing for progeny test is eliminated. This scheme has particular usefulness in breeding for resistance against leaf diseases which appear before flowering and allow identification and intercrossing of resistant plants.

b) *Recurrent selection for general combining ability*

In this scheme, a number of desirable plants are selected in the source population. These plants are selfed and also crossed with a heterozygous tester to identify individuals with good general combining ability. The selected individuals are propagated from the selfed seeds and they are also intercrossed in all possible combinations. The bulk of seeds of the intercrosses serves as a base population for further selection and improvement. This system capitalises on additive genetic variance and can be employed for producing new inbred lines or commercial composites. One cycle of recurrent selection for gca takes three to four seasons depending upon the technique adopted.

A modification of recurrent selection for gca which takes only three seasons for completing a cycle is also available. In the first season, a number of desirable plants are selected in the source population. These S_0 plants are selfed and also crossed with a heterozygous tester to evaluate for good general combining ability. In the second season, these crosses (top-crosses) are put to yield test and on the basis of performance, S_0 plants are selected. In the third season, the selected S_0 plants are grown from the remnant selfed seeds and intercrossed in all possible combinations. At harvest, equal amount of seed lots from all crosses are bulked. This bulked population serves as the material for the next cycle of improvement.

Recurrent selection for gca may take one more season (that is, four seasons for completion of one cycle) if instead of S_0 plants, S_1 plants are crossed to the tester. In this system, in the first season the selected plants are selfed. In the second season, these selfed plants (S_1) are grown and crossed with a heterozygous tester (used as male here). In the third season, the top-crosses are evaluated and on the basis of the test-crosses performance S_1 lines are selected. In the fourth season, the selected S_1 lines are grown from the remnant seeds (of the first year) and intercrossed in all possible combinations. At harvest, seeds are bulked from all crosses (equal amount) to start the second cycle.

This method of population improvement has been used extensively all over the world, particularly by maize breeders.

c) *Recurrent selection for specific combining ability*

This breeding procedure was proposed by Hull in 1945. In the pattern of operation, this procedure is similar to recurrent selection for general combining ability. The difference is that the tester is selected to provide a measure of specific combining ability also. The ideal choice would be that the tester is a stable, permanent inbred line, particularly if the objective is to develop inbred lines that would combine well with the inbred tester. Where the objective is to develop double top-cross and/or a double cross, the relevant single crosses can be used as testers to identify the best combining inbred lines. This method is based on the assumption that heterosis results from non-additive gene action mainly of dominance or over-dominance type.

d) Reciprocal recurrent selection

The reciprocal recurrent selection procedure was proposed by Comstock *et al.* (1949). It aims at simultaneous improvement of two heterozygous and heterogeneous populations which may be designated as A and B. Population A serves as tester for B and B serves as tester for A. This method is believed to be as effective as recurrent selection for gca if additive effects are of importance and as effective as recurrent selection for sca when non-additive effects are of major importance.

The scheme for reciprocal recurrent selection is outlined below:

First season: Individually selected plants of population A are self-pollinated and out-crossed to plants of population B as tester. Similarly, selected plants of B are self-pollinated and out-crossed to plants in A as tester.

Second season: The test-crosses of populations A and B are evaluated in replicated yield trials. From each trial, on the basis of mean performance, superior progenies are identified.

Third season: Selfed seed of plants selected on the basis of their performance in test-cross are planted in crossing blocks; one block each of plants from populations A and B. Within each block all possible intercrosses are made. At harvest, all crosses of each block are bulked. These bulks will now be known as A′ and B′; A′ being the improved version of A and B′ being the improved version of B. This completes the first cycle of reciprocal recurrent selection. If desired, a second cycle of reciprocal selection can be initiated with A′ and B′.

The main advantages of this procedure are: (1) it is effective regardless of the type of gene action involved; (2) it provides for successive increments of yield increases corresponding to changes in gene frequencies; and (3) the rate of inbreeding can be controlled to maximise the utilisation of initial genetic variability.

Breeding methods for specific purposes

Two additional breeding procedures are occasionally used in cross-pollinated species under special circumstances and for specific purposes. These methods are equally effective for hybrid breeding and population improvement. Since the operational details of these methods are available in most textbooks, in the following the treatment is confined to the special features of their usefulness.

Backcross method: This procedure is a special case of pedigree method by which specific desired gene(s) can be transferred to an otherwise agronomically superior genotype. As in pedigree method, detailed records must be kept to identify recurrent parent, donor characteristics, generations of backcrossing and the expression of the desired traits under transfer. The specific gene(s) may relate to disease resistance or to specific character like protein or lysine content (*opaque 2*) or liguleless. The donor genotype is the non-recurrent parent

whereas the elite genotype or line is the recurrent parent. In cross-pollinated species, backcross method is also used to transfer quantitative traits especially grain yield. Convergent improvement for improving the performance of an inbred line (as described earlier) is a kind of backcross breeding.

In the population improvement approach, backcrossing is used for incorporating exotic germplasm into adapted population to increase its genetic variability and to enhance the opportunity for further selection. The choice of whether the recurrent or the non-recurrent parent is used as male assumes great importance when backcrossing is used in association with half-sib procedure of population improvement. In the half-sib method, where pollination is usually open, the donor parents (a variety, a population, a composite etc.) are planted as sub-populations in the half-sib nursery. It is essential that these be used as females (that is, they are not allowed to shed their pollen). This precaution is necessary to avoid contamination of the main half-sib population (back up population). In the population backcrossing system, the donor parent is used as recurrent parent, so that it is converted into a sub-population of the main population, and possesses all its characteristics in addition to the desirable genes or traits of the original donor parent. The details of the conventional backcross method for the incorporation of a dominant or recessive gene is available in Allard (1960).

Poly-cross method: Tysdal *et al.* (1942) suggested the term poly-cross to represent the progeny of a line which has been out-crossed to several other selected lines growing in the same nursery under open-pollination. This method is mainly used for forage breeding. Selected lines or clones are planted in isolation in several replications to increase random mating. Seeds of the same line from different replications are bulked together. In the poly-cross test next season, these crosses are planted in replicated trials. The promising clones or lines are then selected. These may be used to develop a synthetic or adopted in hybrid breeding.

Poly-cross can be considered as half-sib method of population improvement and the test is essentially a top-cross test for general combining ability.

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4

Breeding a Cross-Pollinated Crops: Pearl Millet

O.P. Govila

1) Origin and distribution

Pearl millet (*Pennisetum americanum* (L) Leeke, 2n-14) originated in west Africa from where it was introduced into India. Large variability is still found in Africa. Pearl millet is a dual purpose crop; its grain is used for human consumption and its fodder as cattle feed. It is cultivated mainly in Africa and Asia, primarily as a grain crop in an area of 26 million ha with a production of 13 million tonnes. As a poor man's bread, it sustains a large population of Africa and Asia. It also contributes to the economy of countries like the U.S.A. where it is grown as a forage crop.

In India, Pennisetum americanum (bajra) is cultivated on 11 to 12 million ha land with a production of about 6 million tonnes. It is grown on marginal lands and in areas of scanty rainfall. Rajasthan, Gujarat, Maharashtra, Uttar Pradesh and Haryana account for about 90 per cent of the total area under this crop.

2) Taxonomic status and wild relatives

The generic name *Pennisetum* has been derived from two latin words *penna,* meaning feather, and *seta,* meaning bristle. It adequately describes the characteristically plumose or feathery bristle of some species. Terrell (1976) has logically argued why *Pennisetum americanum* (L.) Leeke is the correct binomial for pearl millet. Other, previously used names of pearl millet include *P. glaucum, P. typhoideum, P. typhoides,* and *P. spicatum.* It is also referred to by generic names such as *Panicum, Holeus, Setavia, Penicillaria, Chamaeraphis* and *Chaetochloa.*

Pennisetum is a polybasic genus comprising heterogeneous assemblage of about 56 species with chromosome numbers in multiples of $X = 5, 7, 8$ and 9. Examples of species with each of these apparently basic numbers are *P. ramosum*

(*In* = 10), *P. americanum* (2*n* = 14), and *P. purpureum* (2*n* = 28); *P. massaicum* (2*n* = 16, 32); *P. orientate* (2*n* = 18, 27, 36, 45, 54) and *P. squamulatum* (2*n* =54). Pearl millet is the most important constituent of the genus *Pennisetum*.

Several wild species have been used in crosses with pearl millet to economic advantage. *Pennisetum purpureum* (2*n* = 28), commonly known as Napier grass, has been crossed with pearl millet (2*n* = 14) in India and the U.S.A. and triploids so produced have yielded selections of perennial fodder varieties (Burton 1944). *P. squamulatum* (2*n* = 54) is an important drought and cold resistant grassland species which is also a genetic apomict. It has been hybridised with pearl millet to evolve a fodder type, combining the forage quality of pearl millet with the frost resistance and perenniality of *P. squamulatum*.

Similarly, *P. orientate* (2*n* = 36) hybrids with pearl millet have been a good source for fodder improvement and transfer of apomixis (Dujardin and Hanna 1983; 1985). *P. dubium* (2*n* = 66) and *P. mosaiccum* possess high fodder production potential. Pearl millet crosses with *P. setaceum* (2*n* = 27) have been utilised to introgress male sterility genes into pearl millet (Hanna, 1979).

Among the intergeneric crosses, buffel grass, *Cenchrus ciliaris* (Syn. *Pennisetum ciliare* (L.) Link, (2*n*= 36) was utilised in crosses with pearl millet. *Cenchrus ciliaris* produces excellent, highly nutritious fodder before flowering. It is also among the most important perennial pasture species for the semiarid regions of Northern India (Read and Bashaw 1974).

3) Floral biology

Pearl millet is an erect tillering annual which can grow several metres high. Branches may arise from nodes; each branch terminating in an inflorescence. The appearance of flag leaf or boot leaves marks the end of vegetative phase.

Pearl millet is predominantly protogynous and hence highly cross-pollinated. The white styles protrude irrespective of whether the panicle is still inside the boot or is partially or fully emerged. The time taken for the emergence of stigma varies from 12 to 24 hours. Stigmas remain receptive for one to two days after emergence depending upon the variety and weather conditions. Anther emergence starts from the apex of the panicle and proceeds towards the base. Anthesis follows the same course. When the first wave of anther emergence from hermaphrodite flowers has reached the basal spikelets, the second wave from staminate flowers starts two or three days after the first style has emerged.

Pearl millet does not require emasculation for making crosses. The panicles are covered with butter-paper bags at the boot leaf stage. On reaching the protogynous stage, the entire panicle is dusted with the desired pollen ensuring

that no stigma remains unpollinated. When the panicle reaches the anthesis stage, the pollen produced is of no consequence as the stigmas have almost lost their receptivity because of germination of pollen from the earlier pollination. For selfing, the panicle is merely covered with a paper bag.

4) Breeding objectives

4.1 General

The major objectives for pearl millet improvement for grain and forage include improved adaptation, increased drought tolerance, ability to put forth quick growth and increased resistance to economically important diseases and pests.

4.2 Breeding for grain yield

Well-filled compact panicles long earheads, more number of tillers, heavy grains and uniformity of ripening have been the main criteria used in breeding for high grain yields of pearl millet (Krishnaswamy, 1962). Photoperiod insensitivity and early maturity are essential for successful growth of pearl millet in areas where adequate moisture is available for short periods. Early maturity is also helpful for growing more than one crop a year when moisture conditions are favourable.

Dwarf varieties will have an advantage as they permit application of fertiliser, irrigation and other practices of intensive agriculture. They will also be useful for mechanised harvesting and manual threshing. Where pearl millet is grown as a dual purpose crop, it is necessary to give adequate attention to acceptability of the fodder by the animals and its digestibility.

Damage from bird attack may be reduced by breeding bristled varieties. Improved grain quality can be achieved by incorporating yellow endosperm (to improve Vitamin A content) and white endosperm (to improve protein content) characteristics in the grain. The acceptability of these changes, however, will depend upon consumer preferences for colour and texture of the grain.

4.3 Breeding for forage yield

For developing pearl millet for fodder the main emphasis is on increasing leafiness, forage quality and digestibility. The ultimate criterion for assessing forage quality is its ability to support animal growth and productivity in terms of meat and milk. Short day and photosensitive varieties are preferred because they remain vegetative for longer periods, give more uniform seasonal distribution of forage, and are easier to manage when grazed. An easy method to increase leafiness, and hence forage quality, is to breed dwarf varieties with reduced stems.

4.4 Breeding for disease resistance

Sclerospora graminicola (Sacc) Schroet, commonly known as downy mildew, causes the major damage to pearl millet. It wiped out high yielding genotypes in the early seventies and again in the eighties. Therefore, breeding varieties with durable resistance to downy mildew has become the most important objective of pearl millet breeding. Knowledge of genetics of downy mildew resistance and identification of suitable genotypes (restorers and male sterile lines) are essential for sustained production of high yielding hybrids. Similarly, breeding for resistance to ergot (*Claviceps fusiformis* Loveless), smut (*Tolyposporium penicillariae* Bref) and rust (*Puccinia penniseti* Zimm) deserve attention and effort.

5) Collection, maintenance and evaluation of germplasm

Germplasm collection in pearl millet was initiated by the Indian Council of Agricultural Research and Rockefeller Foundation. A world collection of germplasm of different millets was assembled and evaluated by the Indian Agricultural Research Institute, New Delhi. The collection was planted and evaluated at four locations, representing abundant and sparse rainfall, in Northern and Western India. Most lines from Africa were found to be susceptible to rust, smut and downy mildew. Alleles for early flowering (55 days) were found in the east African collection and in some lines from Ghana. Late flowering types (80 days or more) were observed in the west African material particularly from Mali and Senegal. Bold seeded lines were mostly from Kenya, Mali, Sudan and Senegal (Murty *et al.*, 1967).

After the establishment of International Crops Research Institute for Semiarid Tropics (ICRISAT) at Patancheru near Hyderabad, germplasm collection and evaluation received a major boost. ICRISAT is one of 13 global centres, established under the Consultative Group on International Agricultural Research (CGIAR), charged with the responsibility of supplying germplasm. More than 17,000 accessions from 39 countries have been assembled at ICRISAT up to December 1984. In addition, 68 wild species and some interspecific hybrids are also maintained.

Evaluation of this germplasm has identified lines resistant to disease, drought and insects and accessions good in quality characters. Such lines can be maintained in isolation growing adequately large plant populations to retain all genes. However, maintenance of a large number of lines in this way will require vast resources.

Varieties possessing genes of recognised value (such as resistance to a specific disease) can also be selfed or sibbed and selection can be imposed to retain the

desired character. Varieties with similar characters, such as maturity or height, can be grouped and multiplied in isolation to make a gene pool for maintenance and use.

6) Breeding for improved yield

6.1 Single plant selection

Many varieties of bajra have been developed by single plant selection. The selection criteria were early maturity, synchronous tillering and compact and good ears of desired size. Generally, selections were made from local varieties (Krishnaswamy, 1962). For example, individual plant selection in 'Akola Bajri' led to the development of three improved cultivars Nos. 37, 54, and 59 (Pandya *et al.*, 1955). Similarly, CO2, CO3, AKP-1, AKP-2, RSJ, RSK, Punjab Type 55, and A 1/3 (Uthkhede, 1972) are selections from existing varieties. Besides indigenous material, variability introduced from Africa has been helpful in developing varieties. For instance, Babapuri and Jamnagar giant in Gujarat, AF-3 in Maharashtra, improved Ghana (renamed Pusa, Moti) in Delhi and S-530 in the Punjab developed from selections made in introduced material are released for commercial cultivation (Athwal and Rachil, 1963). However, the yield potential of these varieties could not be maintained due to extensive outcrossing with local land races.

6.2 Mass selection

Mass selection is an effective method of improving characters with high heritability. This method has unconsciously been used by cultivators in maintaining their varieties and has played an important role in the evolutionary development of bajra plant both in Africa and in India.

6.3 Development of hybrids

Exploitation of hybrid vigour is one of the most efficient means of crop improvement. Like in maize and sorghum, use of this concept has led to a phenomenal rise in yield of pearl millet.

Work on pearl millet hybrid production was started in India nearly four decades ago but without conspicuous impact on grain yields. A limiting factor was the lack of male sterile lines for convenient hybrid seed production. The seed production of early hybrids had to rely upon the protogynous nature of the crop. The hybrids so developed were released for cultivation in the States of Madras (Rao *et al.*, 1951) and Maharashtra (Chavan *et al.*, 1955).

The discovery of cytoplasmic male sterility by Burton (1965) fulfilled the need for a viable and economic method for producing high yielding grain hybrids on a

commercial scale. Burton (1958) identified cytoplasmic male sterility in 1955-1956, in the cross 556 × 23. Two of the four selected plants failed to shed pollen or set seed when selfed. When these two male sterile plants were pollinated (backcrossed) with parent 23, the F_1 progeny again failed to shed pollen on selfing. However, when pollen from other lines was used, the seed set was complete. Burton then crossed 41 inbred lines of female sterile plants (556 × 23) × 23. Six of the resulting F_1 progeny were male sterile, eight others were partially male sterile, and 27 were male fertile. This observation confirmed the existence of cytoplasmic male sterility in pearl millet. It also showed that male fertility could easily be restored. The first CMS line, Tift-23A, was developed using Tift-23B as recurrent parent and was released in 1965 (Burton 1965).

Burton and Athwal (1967; 1968) ascribed the cytoplasmic male sterility to homozygous recessive alleles of nuclear genes, ms_1 ms_1. Such lines are termed 'A' lines. In 'normal' (N) cytoplasm, the recessive homozygote msl msi does not cause male sterility since its genetic effect is not expressed. Such lines are called maintainers or 'B' lines, since as they are used to pollinate 'A' lines, the resulting progeny is male sterile. This mechanism maintains male sterility and allows production of large quantities of seeds of male sterile plants which form the basis for commercial hybrid seed production.

The presence of a dominant MS allele causes male fertility regardless of the type of cytoplasm. Lines which are homozygous for MS_1 MS_1 MS_1 (MS_1 MS_1) are termed restorers (R-lines). When the 'A' lines are pollinated by restorers the resultant progeny is of MSI msl heterozygotes and is real hybrid with restored male fertility.

By this process, several hybrids were produced and released for cultivation (Table 1). The yields of hybrids were 73 to 75 per cent higher than those of the checks based on an average obtained over 17 locations (Anonymous, 1980).

Table 1: Performance of pearl millet hybrids released in India (1965-1972)

Hybrid	× pedigree	Year of release	Centre where developed
HB-1	Tift-24A × BIL3B	1965	Ludhiana
HB-2	„ × J88	1967	Jamnagar
HB-3	„ × J104	1968	Jamnagar
HB-4	„ × K-560	1968	Kanpur
HB-5	„ × K-559	1972	Kanpur/Delhi

Among these hybrids, HB-3 became very popular in areas of scanty rainfall in the country and HB-4 elsewhere. The highest yield recorded was 70 q/ha, with per day productivity as high as 65 kg. The mean performance of HB-1, HB-3 and HB-4 was 29, 33 and 38 q/ha respectively. However, the grain yields ranged

from 16 to 30 q/ha only under rain-fed demonstrations. The hybrids soon became very popular with the farmers, so that beginning with an area of 20 ha during 1966, as national demonstrations, the hybrids occupied 2.2 million (21.4 per cent of the area under Bajra) ha in 1972. The overall grain yields which were stagnant at 3.7 million tonnes rose quickly to 8 million tonnes by the year 1970-71.

The HB series of hybrids, based on the male sterile Tift-23A (with Al cytoplasm), became susceptible to downy mildew during 1970. The first reports of the disease came from Delhi and Haryana but in the subsequent years the disease spread throughout the country wiping out all the hybrids. The cause of this debacle was the genetic uniformity of hybrids and the vulnerability of the common female parent Tift 23 A to downy mildew.

To overcome this problem, efforts were initially directed at inducing resistance to downy mildew in Tift-23A and 'B' through irradiation of 23B seeds and mutation breeding. A large number of plant (A) × (B) pairs were screened for downy mildew resistance. Three 'A' line progenies at M6 generation were found to be free from downy mildew incidence and were stable for male sterility. One of these, MS-5071A showed a high degree of field resistance to downy mildew. Using this line, it became possible to reconstitute the original commercial hybrids. For example, HB-3 (Tift 23A×J-104) was reconstituted and called NHB-3 (5071A-J104). Likewise, HB-4 (Tift 23A×K-560) was renamed NHB-4 (5071A×K-560). However, reconstituted hybrids also had a short life because CMS 5071A also became susceptible to downy mildew and so did the hybrids based on this line.

A male sterile line L-111A, developed at the Punjab Agricultural University Ludhiana (Gill *et al.*, 1975) was also utilised for producing hybrids. During 1976, PHB-10 and PHB-14 were released. However, these failed to make any impact in the farmers' fields. Seed production of hybrids based on MS-L 111A ran into problems because of high pollen shedding in MS-L 111A.

Attempts to breed resistant male sterile lines, using different sources (Boroda 4 and Kano 2457), have been pursued at the Indian Agricultural Research Institute, New Delhi. MS-5141A and MS-5054 (based on Tift 23A cytoplasm) were developed through backcrossing under artificial epiphytotic conditions (Pokhriyal *et al.*, 1976). The availability of downy mildew resistant male sterile lines enabled the generation of a number of hybrids which were tested for grain yield and disease resistance in the All India Coordinated Millet Improvement Programme from 1976 onwards and several hybrids were released for cultivation in subsequent years (Table 2).

Table 2: Performance of downy mildew resistant hybrids (Anonymous 1980)

Hybrid	Parentage	Grain yield (kg/hr)		Year of release	Agronomic attributes
		Av.	Highest		
BM-104	MS-514JA × J-104	3000	4300	1978	Matures in 80 to 85 days suited to both dry and adequate rainfall conditions.
BK-560	MS-5141A × K-560-230	3430	4700	1978	Matures in 85 days suited to adequate rainfall conditions.
CM-46	MS-5054A × D M-46	3579	4125	1982	Matures in 75 days suitable for drought prone areas.
CJ-104	MS-5054A × J-104	3050	3950	1977	Highly suitable for dry areas in Gujarat State. Matures in 80 days.

The hybrids BJ-104 and BK-560 became very popular in India and occupied the entire hybrid growing area of the country.

Diversification of cytosteriles in pearl millet has also been researched on at ICRISAT. In this programme, lines, MS-81A and MS-834A, with good general combining ability have been developed. New hybrid combinations based on these lines are under testing. There is still need for developing downy mildew and early maturing male sterile lines with good general combining ability.

7) Population improvement

7.1 Composite varieties

The first successful attempt to develop composite varieties was made by ICRISAT through recurrent selection (Andrews *et al.*, 1985). Changes in gene frequencies in a positive direction for important characters for all the populations studied were achieved. Genetic gains for grain yield from 1.8 percent (super severe composite) to 5.0 per cent (D 2 composite) per cycle, equalling net gains of 200 to 400 kg/ha per cycle, have been obtained (Table 3). A high degree of downy mildew and smut resistance was also obtained over cycles of selection.

A new variety WC-C75 (ICMV-1) was developed from the 'World Composite' by recurrent selection (Andrews *et al.*, 1985) and was released by the AICMIP in 1982. The performance of WC-C75 is shown in Table 4. The world composite random mating population was developed in Nigeria, in 1971, at the Institute for

Agricultural Research, Ahmad Bello University. It was constituted from derivatives of numerous crosses between diverse sources of pearl millet germplasm and Nigerian early maturing land races, locally known as 'Gero' millets. Full-sib recurrent selection was conducted on the world composite at ICRISAT. During this selection, 441 full-sib families, derived from selected, heterozygous plants in the previous generation were tested at Coimbatore, Hissar and ICRTSAT Centre. Seven superior full-sib families were selected at Coimbatore using supporting data from the other two locations. In the same season, disease free plants from the seven full-sib families were selected from the downy mildew screening nursery at ICRISAT Centre. The resulting SI bulk was sown in the next season's downy mildew nursery, and bulk pollen was used to enforce intermating. The variety produced by this intermating was named WC-C-75.

Table 3: Improvement through recurrent selection in six pearl millet composites

Composite	No. of cycle	Grain yield/cycle		Gain per cycle (%)	Downy mildew incidence (%) Cycle		Gain per cycles
		Co (tonnes ha^{-1})	Latest (tonnes ha^{-1})		(Co.	Latest	
Super severe	5	1.93	2.11	1.8	2.1	1.0	-0.2
New elite	3	2.36	2.57	2.6	1.4	0.0	-0.5
Inter varietal	4	2.11	2.39	1.9	1.9	1.6	-0.1
Medium	5	1.88	2.28	3.2	4.2	3.1	-0.2
Early	4	1.89	2.26	4.1	5.6	4.6	-0.3
D2	2	1.97	2.27	5.0	7.5	4.9	-1.3

From Andrews *et al.*, 1985

Table 4: Grain yield, and downy mildew resistance of pearl millet variety WC-C-75 in all India tests from 1977 to 1983

	1977	1978	1979	1980	1981	1982	1983	Mean	As Percentage of BJ 104
Yield (tonnes ha)	(27)	(33)	(23)	(27)	(30)	(24)	(30)		
WC-C-75	1.63	2.07	1.76	1.85	1.99	1.85	1.79	1.88	101
BJ104[c]	1.79	1.97	1.81	1.81	2.09	1.88	1.68	1.87	100
Local	1.36	1.92	1.61	1.47	1.42	1.86	-	1.61	86
Trial mean	1.54	1.95	1.68	1.73	1.92	1.78	1.69	1.76	94
Downy mildew (%)									
WC-C-75	2.2	2.5	0.9	3.6	—	2.2	0.0	1.6	16
BJ104	8.3	9.8	13.7	8.6	8.1	14.9	8.5	10.3	100

From Andrews *et al.*, 1985

7.2 Synthetic varieties

A synthetic population is defined as one consisting of homo and heterozygotes in polymorphic equilibrium capable of reconstitution, in principle. The initial gene pool of a synthetic is constituted by pure inbreds. These inbreds are selected for superior general combining ability so that, when pooled, intensive matings among them can generate productive progeny. The population is then homogenised in isolation by extensive random mating through generations.

The parental lines of the synthetics developed at ICRISAT comprised Indian ×African crosses, between land races and elite inbreds. As a result of continuous effort, many progenies were produced in various breeding projects. All these progenies have been screened in the downy mildew nursery at the F4 stage and subjected to combining ability tests. Groups of best combiners were selected, random mating or diallel crossing of the selected set of lines was done in isolation to generate a synthetic variety. Further testing was done in the downy mildew nursery. Nearly 500 F_1's, 300 F_2's, 1000 to 2000 F_3-F_5 progenies, and up to 500 F_6, F_7 or inbred lines were evaluated yearly to identify superior parents for producing synthetics (Andrews *et al.*, 1985).

A synthetic variety, ICMS-7703, was thus generated using the following seven inbreds derived from India×African Crosses: (1) (Souna D2×Ex Bourm-2); (2) (J25-1×700515-9-2-3); (3) (B282×J804)-l-3; (4) (J25-1×700797)-5-3; (5) (J-260-1×700557-1)-4-9; (6) (J1798×700594)-2; and (7) (700250×Ex Bornue)-6. It was recommended for release in India in 1984.

8) Future challenges

International collaboration and cooperation is strongly needed to collect the unexploited resources of germplasm, which in turn will be helpful in the development of gene complexes as reservoirs of variability. Development of early maturing restorers with good combining ability need strengthening. Genetic as well as cytoplasmic diversification of male sterile lines with high resistance to downy mildew disease is needed for developing superior hybrids. Other areas deserving attention are: genetics of resistance against different diseases and devising methodologies for breeding resistant varieties; studies on genetic variation in pathogens and refinement of techniques for screening material for resistance to diseases.

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5

Vegetatively Propagated Crops

N.M. Nayar

A close relationship between the mode of reproduction of a cultivated plant and the methods to be employed for its improvement is now well accepted. Crop plants are broadly placed in two groups according to the mode of their reproduction: sexually reproduced and asexually propagated or reproduced plants. Asexual reproduction does not involve the union of gametes and/or reduction in chromosome numbers.

Plants may reproduce asexually in a variety of ways. In 1980, Mayo has identified the following methods of asexual reproduction on the basis of the classification made earlier by Stebbins in 1941 and Brown in 1972.

Asexual: Vegetative apomixis, yielding no seeds—Fairly common in some groups, *e.g.*, bulbils in onion, carmils in gladiolus, leaf plantlets in Kalanchoe

: Agamospermy, yielding seeds—Adventitious embryony, *i.e.*, type of sporophytic budding

: Parthenogenesis, *i.e.*, female gamete yields a sporophyte without syngamy. *e.g.*, in maize, wheat, tobacco

: Non-recurrent apomixis, *i.e.*, normal meiosis yielding a haploid sporophyte via haploid gametophyte. *e.g.*, in maize

: Agamogony, *i.e.*, a daughter sporophyte develops from a diploid gameto-phyte

: Apomeiosis, *i.e.*, diploid gametophyte functioning in meiosis

: Apospory, *i.e.*, gametophyte formed by meiotic division

: Pseudogamy, *i.e.*, maternally derived offspring after induction by pollination but no gametic fusion, *e.g.*, in potato, strawberry

: Semigamy, *i.e.*, maternally and/or paternally derived off-spring after induction of pollination but no gametic fusion, e.g., in cotton

: Apogamety, *i.e.*, embryo develops from non-egg nucleus

: Polyembryony, *i.e.*, occurrence in the seed of more than one embryo; common in citrus, also in mango, some cassia

In this chapter, grouping is considered into two categories only, vegetative propagation and apomixis. Vegetative propagation involves multiplication of the plant using a somatic tissue such as the root, stem or leaves.

In apomixis, seed is produced, but the embryo develops without fertilisation (*i.e.*, without the fusion of male and female gametes) from one of the unreduced cells of the ovule.

Vegetative propagation includes propagation from: (i) rhizomes, or under-ground stems as in ginger (*Zingiber officinale:* Zingiberaceae), Johnson grass (*Sorghum halepense:* Poaceae); (ii) bulbs as in onion *(Allium cepa:* Alliaceae), tulips (*Tulipa* spp: Liliaceae); (iii) corms as in gladioli (*Gladiolus* spp.: Iridaceae); (iv) tubers, a modified underground stem, as in potato (*Solarium tuberosum.* Solanaceae); (v) runners or stolons as in strawberry (*Fragaria* spp.: Rosaceae); (vi) bulbils as in lilies (*Lilium* spp.: Liliaceae); (vii) suckers as in bananas (*Musa acuminata:* Musaceae); (viii) roots, as in poplars (*Populus alba:* Saliceae), bread fruit (*Artocarpus incisus:* Moraceae); (ix) stem cuttings as in sugar cane (*Saccharum officinarum:* Poaceae), black pepper (*Piper nigrum:* Piperaceae); and (x) leaves as in Bryophyllum (*Brophyllum pinnatum:* Crassulaceae), Kalanchoe (*Kalanchoe blossfeldiana:* Crassulaceae).

As already stated, in apomixis seed is produced, but the embryo develops from an unreduced cell of the ovule. The development of apomictic seed can occur in many ways, but we shall not got into its details. Apomixis is found in a number of forage plants such as Kentucky blue grass (*Poa* spp.: Poaceae), Dallis grass (*Paspalum* ssp.: Poaceae) and many Citrus species (*Citrus* spp.: Rutaceae).

A related form of asexual reproduction is parthogenesis. In this, the egg cell develops into an embryo without fertilisation. The embryo may develop from a cell (when the process is called apomixis), or from a reduced gamete.

From the above account, it would be seen that a large number of crop plants are asexually propagated. These include root and tuber crops (such as potatoes, sweet potatoes, yams, colocasia), sugar cane, many fruit and ornamental plants, some forage plants and many tree species. A majority of the varieties of most asexually propagated plants are also able to reproduce sexually, even though the offspring may be sometimes weak. A sexual reproduction is particularly

beneficial in fruit and ornamental plants where propagation of a particular genotype becomes important for the sake of retaining flavour (as in fruits), or colour of foliage or flowers (as in many ornamentals). There are only very few cultivated plants that are obligately vegetatively propagated.

Since the asexually propagated plants are reproduced by vegetative means genetic segregation and recombination normally do not occur in the successive clonal generations through which it passes while cultivating them. This leads to three major consequences: (i) they gradually accumulate naturally occurring mutations leading to increased heterozygosity; (ii) they progressively accumulate deleterious genes; and (iii) the constant disuse of sex leads to progressively higher levels of male and female sterility. The occurrence of male sterility is more frequent than female sterility.

When the principles of genetics were not known and the science of plant breeding had not been developed/was unknown, improved varieties of several flowering and fruit plants were being produced by identifying or isolating and propagating 'sports', which are naturally occurring variations resulting from mutations at one or more less stable loci. Classical examples of such varieties are the Cayenne variety of pineapple, Russet Burbank variety of potatoes, Anab-e-Shahi variety of grapes and most early varieties of ornamental and fruit crops.

Method of breeding

In the breeding of vegetatively propagated field crops (yams, colocasia, sweet potato, potato, etc.), the techniques employed are essentially the same as those used in the breeding of sexually propagated plants and include introduction, selection, and hybridisation. The differences are only in the handling of the population under testing. In vegetatively propagated plants, once a desirable genotype is identified, it can be maintained indefinitely by asexual or vegetative means, without loss of genetic integrity. This is a great apparent advantage. However, there are also some attendant disadvantages which are discussed here:

i) As successive vegetative generations do not go through the 'genetic filter' of meiosis, the selected clones tend to accumulate systemically transmitted diseases, more particularly, virus and mycoplasmal diseases. They also accumulate mutations, a vast majority of which are deleterious in their effects. To overcome these problems (a) maintenance of elite seed stock in disease-free conditions becomes obligatory; and (b) the seed stock itself should be regularly built by selecting elite, true to type, plants from large clonally propagated populations.

In recent years, however, some of these difficulties have been overcome by maintaining the clones in tissue culture form. While the utility of tissue cultured clones has been established for some crops, for others, the techniques or methodologies that would ensure the genetic integrity of the clone in tissue culture form are yet to be developed. The necessity of evaluation of tissue culture methods for specific crops arise, because while 'sports' occurring in nature during traditional maintenance become visible in the standing crop, and can, therefore, be rouged out, this possibility is not available when clones are maintained in tissue culture form. It is, however, obvious that tissue culture maintenance is more advantageous than the traditional methods.

ii) Maintenance of germplasm required in breeding programmes is expensive and difficult for reasons given above.

iii) Most varieties and cultivars are affected by various degrees of sterility, poor vigour and aborted seeds. This, sterility as has been stated already, is a conseuqence of their not being subject to the 'genetic filter' of meiosis;

iv) Some species, and several varieties of most vegetatively propagated plants, do not produce flowers. Special treatments have therefore to be devised to induce them to flower and to produce seeds. The best example is that of sugar cane in India. It flowers normally in south India, but produces flowers and seeds only shyly in the North.

The size of the breeding populations

Since most varieties of vegetatively propagated crops are heterozygous, the F_1, and not the F_2, generation, constitutes the primary segregating generation. Hence, the size of the F_1 population has to be much larger when compared to that required for autogamous plants. In addition, as a group, a large percentage of vegetatively propagated plants are polyploids in nature (such as sugar cane, potato, sweet potato and bananas) and require raising of a larger population to obtain the desired recombinants.

Since several cultivars of some vegetatively propagated plants would not either flower, or even if they flower, would be affected by higher levels of sterility (more commonly, male sterility), it becomes necessary to produce a larger number of hybrids than generally required in sexually propagated plants.

Broadly, the methods and procedures employed for varietal improvement in vegetatively propagated plants are similar to those used in sexually propagated plants. The differences occur in certain matters of details in that they vary with the end use of the crop, the level of ploidy, and the prevalence of sterility in the species.

A standard procedure employed in potato, a widely cultivated vegetable and food plant, and considered to be an auto-allotetraploid, is given here. Commercial potato has $2n = 4x = 48$ chromosomes. It flowers generally under only the long-day conditions prevailing in the hills, though more than four-fifths of the crop is grown in the Indo-Gangetic plains under shortday conditions prevailing during the autumn-winter months of October-March.

Parents are chosen on the basis of their desirable characteristics in the same manner as is done in any breeding programme. About 25 crosses are made for each programme, and in each cross, a population of about 2500 seedlings is sought to be raised. Thus more than 60,000 seedlings are raised in the first (which is the segregating) generation. Very broadly, it can be stated that in the first three clonal generations, rejection of undesirable genotypes is done, rather than selection, and the selections of the desirable types is done in only the subsequent clonal generations. The remaining details are given in Table 1.

Mutation breeding

Because of the problems posed by non- or poor flowering and sterility, mutation breeding offers very good possibilities in this group of cultivated plants, particularly in ornamental and fruit plants. There are numerous examples of the development of new cultivars in ornamental plants using mutation breeding. The classical work of Zwintscher (1955) in apples and cherry, and Bauer (1957) in currants (*Ribes*) demonstrated the potential of induced mutagenesis as a tool for fruit tree improvement. In spite of this early work, the fruit plants breeders have not made extensive use of this technique. This may be due to the more intensive operation of intrasomatic competition—also known as diplontic selection—in vegetative propagated plants as compared to the seed propagated plants (Nayar, 1970; Broertjes and van Harten, 1978; see also chapter 10 on mutation breeding in this volume). To reduce the operative intensity of intrasomatic selection, it would be advisable to mutagenise the plant propagules when they are in a dormant or semi-dormant condition, as in this condition the number of meristematie cells would be comparatively less. Exposure of stem cuttings to X or gamma irradiation, and repeatedly cutting back the shoots, eliminates the chimeric condition. A successful modification of this method has been developed in potato by Nayar and Dayal (1970). In this, longitudinally cut halftubers were exposed to gamma irradiation—the other half being kept as control, and sprouts emerging from them were periodically removed and planted individually. By this procedure, the frequency of mutant plants recovered in the VM_2 generation could be increased by up to 50 per cent, thus registering a significant increase of mutation rate over that obtained by earlier workers. Similar modifications could be developed for other vegetatively propagated plants

Table 1: Suggested procedure for handling seedlings in a potato breeding programme

Stage	No. of culture	No. of tubersper culture	Criteria for selection
F_1	Plant 62,000 seedlings (25 cross combinations, 2500 seedlings per cross or family): Reject 50,000 seedlings (clones), and retain about 12,000 clones	3-5	The seedlings are raised in the fields under low aphid conditions. At harvest the genotypes showing long stolons, irregular tuber shape, deep eyes, russetting, cracking and undesirable tuber colours are rejected. Yield is not considered for selection at this stage.
F_1C_1	Plant about 12,000 clones: Reject 8,000 clones , and retain 4000 clones.	3-5	The cultures are planted in short observation rows with control varieties after every 30 rows. At full growth, weaklings and diseased cultures, if any, are rouged out. Late blight susceptible and virus infected clones are also rejected. At harvest, further rejections are done on the basis of undesirable tuber types. Within family comparisons of cultures are made for tuber bulking and uniformity in size. The cultures producing larger number of smaller tubers are rejected.
F_1C_2	Plant about 4,000 clones: Reject 3,000 clones and retain about 1000 seedlings	15	Plant the cultures in single observation rows along with control varieties, Inferior ones are further rejected as in F_1C_1 except that the cultures producing more tubers, but of uniform size are retained for further trials.
F_1C_3	Plant 1,000 clones: Select about 100 clones	60	The cultures showing high percentage of rotting, shrinkage and oversprouting during cold storage are rejected. The remaining hybrids are planted in 4 rows of 15 tubers each with 3-4 standard cultures (*e.g.*: Kufri Lalima and Kufri Chandramukhi) as controls. 60 days after planting, one row of each culture is harvested and compared with the early maturing cultivar Kufri Chandramukhi for number of marketable tubers, and firmness of skin and yield. Cultures that are better than control are identified for assessing under early group next year. The remaining 3 rows of all the cultures are harvested at 90/100 days maturity. The cultures that yield at least 10 per cent more than the control variety are retained.

Stage	No. of culture	No. of tubers per culture	Criteria for selection
F_1C_4	Plant about 10 clones:Select 30 clones	240	After taking out the seed material from the cold store, examine and reject those showing poor keeping quality (high percentage of rotting, shrinkage and sprouting) as in the previous generation. The remaining cultures are planted in preliminary yield trials in different maturity groups. Each trial is laid out in randomised block design using 10-15 cultures plus one or two standard cultivars as control in four replications. The plot size consists of 60 tubers planted in 4 rows. After harvest, about half to one-third of the cultures are selected based on results of statistical analysis. The cultures showing hollow heart and such other visible abnormalities are also rejected.
F_1C_5	Plant about 30 clones: Select 10 clones	960	The material taken out of the cold store is examined for storage characteristics, and those that are undesirable, are rejected. Record observations on tuber sprouting and rottage in the cultures retained for further evaluation. The selected cultures are tested in confirmatory yield trials using randomised block design. Each trial may have 15-30 treatments, including controls, and 8 replications. The plot size may consist of 120 tubers planted in 6 to 8 rows. From these trials, the 10 best hybrids are selected. The produce of the selected cultures is then distributed as follows:Agronomic evaluation: 1,000 tubers of each culture and the control cultivar are assessed for yield potential at 3 levels of fertiliser application. These levels will vary with time and locations, but will generally consist of the commonly used dose and about half and double of the recommended dosage levels. The trials will continue for 2-3 seasons. The agronomist will maintain the seed stocks for the next year's trials. Physiological and biochemical evaluation: 1,000 tubers of each culture are used for keeping quality tests and 100 tubers each for estimating tuber dry matter, true protein, total sugars, reducing sugars, starch,

Stage	No. of culture	No. of tubersper culture	Criteria for selection
			phosphorus, potassium, vitamin C and glycoalkaloids. Selections are tested for chipping quality also. Pathological evaluation: 100 tubers of each culture are used for screening against late blight in foliage and tubers, early blight, wart, common scab, black scurf, storage diseases like dry rot, soft rot and charcoal rot, and the viruses X, S and Y. Entomological and nematological evaluation: 50 tubers each are used for testing against tuber moth, root knot and cyst nematodes.
F_1C_6	Plant 10 clones: Select 4-5 clones	3,750	The confirmatory yield trial is repeated as in previous stage. The remaining material is used for multiplication. Keep only the top 4-5 high yielding cultures for next year's trials.About 10,000 tubers each of all selected cultures are used for laying out 4-5 regional adaptability trials under three fertility levels. The scope and extent of these trials are to be finalised.
F_1C_7	Plant 4-5 clones: Retain all the 4-5 clones	5,000	Plant the hybrids in multiplication blocks. Repeat the trials as in F_1C_6 stage. Mark 100 healthy and true to type plants for giving the produce to seed certification unit. The harvest of each selected plant is kept separate for this purpose. Morphological and other characters of each of the selected cultures are recorded. At harvest, the results from different evaluations and 4-5 regional trials are expected to be available. Two thousand tubers of each of the selected cultures along with culture descriptions are supplied to the Seed Preparation Unit of All India Coordinated Potato Improvement Project.
F_1C_8	Plant 4-5 clones: Retain all the 4-5 clones	4 q	The trials and multiplication are continued as in F_1C_7 stage.
F_1C_9	Plant 4-5 clones: Select the best 1-2 clones	20 q	The trials are continued as in F_1C_8 stage. Based on the results obtained in the three seasons from the multilocation and other trials, one or two of the best cultures are identified for release.

Source: Gaur *et al.*, 1984.

also. For a detailed discussion on all aspects of mutation breeding in vegetatively propagated plants, the reader is recommended the book by Broertijes and van Harten (1978).

Another method of overcoming intrasomatic selection is to treat cultured tissues with mutagens, isolate individual cells and induce them to grow into plants. This is no doubt a tedious procedure, but ensures the elimination of intrasomatic competition to a better extent. In recent years, there has been an increasing interest in developing this methodology.

Use of biotechnological methods

In vegetatively propagated plants, newer biotechnological techniques like somatic hybridisation including protoplast fusion appears to offer tremendous possibilities, but research would have to go a long way before these methods could be put to practical application. This effort is, however, necessary if varietal improvement in vegetatively propagated plants is to make great leaps forward.

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6

Breeding for Disease Resistance

R.N. Kulkarni and V.L. Chopra

Plant diseases, in spite of the various measures adopted to control them, continue to be a major cause of crop losses. At times these losses have been so heavy that they have caused severe famines. Diseases are caused by a wide variety of organisms such as fungi, bacteria, viruses, mycoplasma and nematodes. The diseases may be partially or completely controlled by chemical, cultural and genetic methods. When no single method is effective in controlling a disease, integrated control constituting a combination of the three methods of disease control (chemical, cultural, genetic) may be effective. If host resistance alone is sufficient to check a disease, then it is to be preferred to other methods because it is the least expensive, has no adverse environmental effects and involves no specific action on the part of the farmer, even when only partial, genetic resistance supplements chemical control and makes it more effective in comparison with chemical control on a completely susceptible cultivar.

Many plant diseases have been successfully controlled by exploiting genetic resistance especially where differentiation in the pathogen with respect to resistance genes in the host is lacking. In some cases, even when pathotype differentiation occurs in the pathogen, genetic resistance in combination with other methods has given adequate control. Breeding for resistance to highly variable air-borne fungal pathogens has claimed much time and effort of plant breeders and success has often been transient. The reasons for the shortlived success have become clear from research during the last quarter of a century.

Nature of resistance

Host resistance may be true resistance that operates when the host and the pathogen come into contact with each other or it may be due to mechanisms, collectively called "avoidance". Avoidance reduces the contact between the host and the pathogen. Well known examples of avoidance are: (1) resistance of barley cultivars with closed flowers to *Ustilago nuda;* (2) resistance of tall

wheat cultivars to glume blotch, *Septoria nodorum* (the height of the plant reduces the spread of inoculum to the upper parts of the plant); (3) resistance of rubber variety LCB 870 to mildew and of coffee cultivars SRG and Dilla to coffee berry disease due to reduced period of susceptibility; and (4) reduced susceptibility of the pole and intermediate types of bean cultivars, compared to bush-type cultivars, to web-blight (caused by *Rhizoctonia solani).* The pole types have a reduced exposure to rain-splashed soil which is the main source of inoculum. The mechanisms of avoidance or escape, if identified, can be used as valuable characters in designing breeding programmes (Hooker, 1980).

True resistance to fungal infection may arise from physical factors such as increased cuticle thickness or distribution of thickened mechanical tissues like sclerenchyma (Day, 1974). It may also be due to hypersensitive reaction in which the invaded tissue is killed and prevents further infection of the host tissue. Some times the resistance is not as clearcut as in the case of hypersensitive reaction, but the growth and reproduction of the pathogen is considerably restricted compared to that on a completely susceptible cultivar.

Kinds of resistance and aspects of terminology

Resistance

Resistance is a state of less disease with immunity (no disease) representing the extreme (Simmonds, 1983). Resistance and susceptibility are relative terms. All varieties that fall in between the extremes of the resistance scale, that is, immunity and extreme susceptibility (which is unknown), are partially resistant and partially susceptible. The designation of a given variety as resistant depends on the susceptible standard used.

Race

Race is a "taxon" within a pathogenic species which is characterised by a specific combination of virulence genes (Zadoks, 1966). The traditional system of naming races of cereal rusts, was based on assigning numbers and letters depending on their reaction on an arbitrary set of differential varieties. There was neither any descriptive meaning in these names nor was there a genetical relationship between consecutively numbered races. Isolates which gave the same reaction on the standard differential hosts, but differed on other varieties, were called subraces or biotypes within a race. A major disadvantage of this method of naming races was that the letter or the number of a given race provided no direct information on the resistance genes effective or ineffective against it. The system of designating isolates of the cereal rust pathogens by their virulence/avirulence formulae was therefore, proposed by Green (1965)

and is now being increasingly used in literature. The earliest system of designating races by their virulence genes was developed for potato late blight fungus (Black *et al.*, 1953).

Virulence

Virulence is the ability of the pathogen to overcome resistance of the host. Generally, virulence confers ability on the pathogen to attack some genotypes of the host but not others. Virulence is the counterpart of vertical resistance in the host. It is also called vertical pathogenicity. Virulence is generally mono- or oligo-genically inherited.

Aggressiveness

Pathogenic strains are said to differ in agressiveness if they can cause different amounts of disease on a given host genotype, even while carrying the same virulence genes. Aggressiveness is also called horizontal pathogenicity, because effects of increased horizontal resistance can be counteracted by effects of increased aggressiveness. Agressiveness is believed to be quantitatively inherited.

Non-specific major gene resistance or unclasssified resistance

In a majority of plant pathogens, no specialisation has been found in respect of resistance genes in the host. Disease resistance against such pathogens is generally governed by mono- or oligogenes and is stable. Examples of diseases against which such resistance operates are: milo disease of *sorghum,* caused by *Periconia circinata;* cabbage yellows of tomato, caused by *Fusarium oxysporum conglutinanus;* bacterial wilt of tobacco, caused by *Pseudomonas solanacearum;* Victoria blight of oats, caused by *Helminthosporium victoriae;* raspberry ring spot caused by tomato ringspot virus and potato viruses X and Y (Simmonds, 1983; Vanderplank (1984). In the absence of variation for pathogenicity in the disease causing organism, the resistance cannot be classified as vertical or horizontal (see below).

Vertical resistance (VR)

Vertical resistance confers resistance to some races of the pathogen but not to others. A gene-for-gene relationship is involved in VR, that is, a gene for resistance in the host is matched by a gene for virulence in the pathogen. A minimum of two differentially interacting host and pathogen genotypes are required to infer the existence of VR or the gene-for-gene relationship (Table 1).

Table 1: Differential interaction between two genotypes of the host and two genotypes of the pathogen

Virulence gene	Resistance gene	
	R_1-	R_2-
v_1v_1(in race 1)	Susceptible	Resistant
v_2v_2 (in race 2)	Resistant	Susceptible

When the resistance is not qualitative (that is, expressed as resistance/susceptibility) but is quantitative, then VR can be inferred from the significance of variety × race interaction term in the analysis of variance table. VR has been found in all classes of pathogens namely, viruses, bacteria, fungi, nematodes, insects, angiosperms (*Orobanche-Helianthus*). VR, when complete, acts by reducing the initial inoculum.

Horizontal resistance (HR)

Horizontal resistance is effective against all the races of the pathogen. For this reason, it is also called non-specific resistance. It is characterised by the absence of differential interaction between host varieties and pathogenic races. It can also be inferred from the constant ranking of cultivars irrespective of the pathogenic race. For example, if variety A is more resistant than variety B against race 1, it is also more resistant than variety B against any other race of the pathogen. HR acts, mainly, by reducing the rate of disease increase. HR is said to be durable because it does not exert selection pressure on the pathogen.

The terms VR and HR were first developed by Vanderplank (1963) to apply to those host-pathosystems which exhibited genetic variations for resistance and pathogenicity. He classified all variations for resistance in the host into VR and HR, and all variations for pathogenicity in the pathogen into virulence and aggressiveness. The classification was essentially a statistical one (similar to the classification of quantitative genetic variation into additive and non-additive variations). All variations which manifested as the main effect of the host in the analysis of variance table were attributed to HR while the interaction effect was considered to be due to VR. Similarly, variations for pathogenicity were classified into those due to aggressiveness and virulence which manifest as main effects and interaction effects respectively, of the pathogen in the analysis of variance table.

Durable resistance

Durable resistance is one that remains effective during prolonged and widespread use in an environment favourable for disease development (Johnson, 1984).

The test of durability is the test of resistance over time and extensiveness of cultivation. Durable resistance thus can be inferred only on retrospective analysis. The concept of durable resistance includes no implication about genetic control of resistance, or its mechanism, its degree of expression or its race specificity. In the absence of a valid criterion of selection the breeder has no way of knowing whether the plant he selects has durable resistance. The procedure recommended for breeding for durable resistance (Johnson, 1984) is similar to the one recommended for breeding for HR, except that it starts with the cultivars which have exhibited durable resistance.

Tolerance

A cultivar is said to have true tolerance if it shows a susceptible infection type and supports the same amount of pathogen as another cultivar but has significantly better yield and quality (each relative to its disease-free check). A cultivar will also be termed tolerant if it has the same yield and quality as another cultivar but supports more of the pathogen (Politowski and Browning, 1978). Tolerance, like resistance, is a relative term. However, it is not resistance because it does not restrict the growth of the pathogen and, therefore, does not exert selection pressure on the pathogen. Assessment of the actual amount of disease is a major practical problem in detecting true tolerances. Gaunt (1981) suggested that the following points should be taken into consideration while detecting true tolerance:

1) Disease measurements should be made by several different parameters and not by a single parameter like disease severity or sporulation per unit area, as is usually done. This is because a single parameter does not generally reflect the total effects of the disease.
2) Disease measurements should be made during the entire period of epidemic so that the effect of rate-reducing resistance could be identified.
3) Studies should be conducted in cropping situations and not in single row or hill plots because competition among plants is an important factor in crop loss-disease interaction studies.
4) Yield should be measured as final harvested weight per unit area. If possible, quality should also be taken into account.
5) Potential yield should be equivalent to that of the non-tolerant cultivar.

Detection and measurement of resistance

Resistance to disease can be detected by evaluating plants in the field under natural conditions of disease development or by subjecting the test plants, or plant parts, to artificial inoculation.

Screening for resistance in the field under natural conditions is generally done when artificial methods of inoculation are not available or when disease is of minor importance and does not warrant the use of expensive and sophisticated methods. While screening for resistance under natural conditions of disease, it is important that chance escapes are not confused as resistants. To some extent, randomisation and replication of test genotypes in the field helps in minimising the chances of misclassification of resistance. Tests, repeated over time, help in improving the reliability of evaluation. It is also important to distinguish between avoidance and resistance since avoidance mechanisms, like resistance, can be environment specific. Selections of good locations for testing for resistance is very important. Such locations are those where disease is endemic and the pathogen is genetically highly variable. For example, the choice of Toluca valley in Mexico for testing potato for HR to late blight by Rockefeller Foundation is considered to have done more than any other thing to promote an understanding of potential of HR in plant breeding (Simmonds, 1979). In India, breeding wheats for rust resistance would not have achieved the success it has, if screening facilities in the Nilgiri Hills were not available.

Screening for resistance in the field under natural conditions of disease has, however, several disadvantages such as, interference from other diseases, fluctuating environmental conditions and their interactions with the host and the pathogen, non-random distribution of the pathogen (especially in the case of soil-borne pathogens), dependency on natural races of the pathogen and on environmental conditions favourable for disease development. Artificial screening techniques have, therefore, been developed especially for important diseases of major crops. A good screening technique should fulfil the following requirements (Hooker, 1980): (1) It must be repeatable. (2) It should identify different gradations of reaction to the pathogen. (3) It should reflect reaction of the host under field conditions. (4) It should be easy to handle. (5) It should be adaptable to breeding and selection programmes of crops. (6) It should screen a large number of plants without demanding much time and space.

All artificial screening techniques involve: (i) increase of inoculum of an appropriate strain of the pathogen on a susceptible host or artificial medium; (ii) inoculation of plants or plant parts by spraying, dusting, injecting, dipping, or mixing in soil; (iii) incubation under optimum environmental conditions; and finally, (iv) evaluation. Care should be taken during the increase of inoculum and subsequent incubation of inoculated plants or plant parts, to eliminate the possibility of contamination with strains of the pathogen other than the one for which screening is being carried out. It is also important that the culture of the pathogen has optimal degree of virulence and aggressiveness. Some pathogens are known to lose virulence and/or aggressiveness when cultured for long periods in the absence of host plants.

Screening for resistance can also be done by exposing test plant to pathotoxins. A classical example of the use of selective pathotoxin for screening for resistance is that of victorin, produced by *Helminthosporium victoriae,* the causal organism of Victoria blight of oats.

Selective media, prepared by a combination of specific chemicals, can be used to promote the growth of specific species of plant pathogens. Species-specific media are now available for some soil fungi and the chemicals used in these media can possibly be used for testing resistance against such fungi. For example, potato pieces soaked in the antibiotics, pimaricin and streptomycin, will pick up infection specifically with *Pythium aphanidermatum* when placed on naturally infested soil (Stanghellini and Kronland 1985). This specificity provides an avenue for testing resistance of different host genotypes to *P. aphanidermatum.* The inoculum level of the soil can then be determined using *P. aphanidermatum* specific medium (Burr and Stanghellini, 1973). The method is rapid, will eliminate artificial culture of the pathogen and thus make it possible to screen a large number of genotypes in the laboratory in a short time.

Artificial disease epidemics can also be created in the field on an experimental scale. Susceptible genotypes are grown as spreader rows among test genotypes. The spreader rows are inoculated with an appropriate strain of the pathogen well in advance of the arrival of the natural inoculum so that the inoculated strain is already present in abundance in the experimental field when the natural inoculum arrives. By manipulating the date of planting, it is ensured that the test genotypes reach the appropriate stage of development when the weather conditions become favourable for disease development. Standard cultivars with known reaction to disease are planted at regular intervals to serve as controls.

While dealing with rate-reducing resistance (resistance that reduces rate of disease development) to airborne pathogens, interplot interference is an important aspect needing consideration. Interplot interference, which can lead to under or over estimation of resistance, can be avoided by conducting tests in large isolated plots. In spite of interplot interference, ranking order of barley cultivars for resistance to leaf-rust was not affected in small plots. However, the difference between the most resistant and the most susceptible cultivars in small adjacent plots was under-estimated by a factor of 100-400 when compared to large isolated plots (Parlevliet and Van Ommeren, 1975).

Measurement of resistance

Qualitative VR is measured in terms of infection types, 0(necrotic or chlorotic fleck with no sporulation) to 4(sporulating pustule without chlorosis). Inter-mediate infection types 1, 2 and 3 represent increasingly sporulating pustules

with necrosis or chlorosis. Further gradations within each infection type are designated by + or – notations. Infection types 0, 1 and 2 are classified as resistant and 3 and 4 as susceptible.

Quantitative assessment of resistance is made in terms of disease severity, that is, proportion of plant tissue affected by disease. Standard disease severity diagrams are available for many of the important diseases, especially of important cereals. These diagrams are used for estimating disease severity visually. Rate of increase of disease (Vanderplank's infection rate) and/or area under disease curve are usually calculated from disease severity data collected over a period of time to quantify the level of rate reducing resistance (or HR, if an appropriate race of the pathogen is used) of a cultivar. The formulae for calculating infection rate and area under disease curve are given below:

$$\text{Infection rate } (r) = \frac{1}{t_2 - t_1}\left(\log_e \frac{x_2}{1-x_2} - \log \frac{x_1}{1-x_1}\right)$$

where x_1 and x_2 are the proportions of disease at t_1 and t_2 (Vanderplank, 1963).

$$\text{Area under disease curve} = \sum_{i=1}^{n}\left[(x_{i+1} + x_i)/2\right]\left[t_{i+1} - t_i\right]$$

where x_i is a proportion of disease at time t_i (Shaner and Finney 1977). The area under disease curve is a more useful parameter for distinguishing differences in levels of rate-reducing resistance of cultivars than infection rate (Kulkarni, 1985).

Many diseases are scored on 0-5 or 0-9 E visual scales. Disease incidence (per cent of plants infected) is used in case of diseases where plants can be classified into only infected and non-infected.

Factors affecting expression and level of resistance

Plant disease is the result of interactions between the host, the pathogen, the environment and the time interval during which these three factors interact among themselves. It is important to recognise the effects of each of the contributing factors individually, as also of their interactions while screening for resistance.

Effects of the host

The genotype of the host plant, its stage of development and its physiological condition influence its response to a given disease. The response of plants to diseases may vary with age because the resistance genes may function at

some stages of plant development but not at others. For example, genes for adult plant resistance to rusts in cereals are effective in adult plants but not in seedlings.

Resistance or susceptibility may be associated with ontogenic stage of the plant. Generally, resistance is maximum during middle age that is, before the appearance of reproductive structures (Bell, 1982). In cereals, slow rusting resistance is known to decline after anthesis. Therefore, when screening for slow rusting resistance under field conditions, comparisons are valid only between lines which have the same or similar maturity periods. Comparison of slow rusting resistance of host genotypes differing in maturity periods can be misleading because of host × environment interaction. For this reason, comparison of determinate and indeterminate cultivars for resistance to foliar diseases on the same calendar date can be misleading (Bell, 1982). This is exemplified by the results of a recent study by Hamblin *et al.* (1984) who compared resistances of four lupin cultivars (differing in maturity period) to *Phomopsis leptostromiformis* at three planting dates. At planting date one, early flowering lines were most resistant while at planting date two, they were least resistant (Table 2). It clearly indicates an interaction between the cultivar, its stage of development and the environment.

Table 2: *Phomopsis* level (per cent) on different lupin cultivars

Planting cultivar date	P 22612 (very early)	Illyarrie (early)	Chittick (mid-season)	Marri (late)
Date 1	43	47	53	59
Date 2	59	53	50	51
Date 3	25	22	22	17

Again, while screening for resistance to ergot and smut in *bajra,* it is important to ensure that no pollination occurs before and after inoculation (Thakur and Williams, 1980; Thakur *et al.*, 1983). This is because infection of these diseases occurs through stigmas and pollination offers protection against infection by inducing rapid withering of stigmas. Failure to recognise this has been the major reason for lack of progress in developing resistance to ergot and smut in *bajra.*

It is clear from the above examples that a failure to recognise the effects of the host, and of the interaction of the host with environment, may lead to incorrect conclusions about the resistance levels of cultivars, especially when the cultivars differ in growth patterns.

Effect of the pathogen

Virulence, aggressiveness and inoculum density of the pathogen are among factors that influence the expression of resistance of a given host. Virulence is

the ability of the pathogen to overcome resistance genes in the host. Therefore, host resistance is expressed only in the absence of relevant virulence genes in the pathogen.

Pathogenic strains with the same genes for virulence can differ in aggressiveness and, therefore, cause different amounts of disease on a given genotype of the host. Thus, if screening for resistance is done with a less aggressive strain, then some loss of resistance is expected when a more aggressive strain gets selected in nature. This loss of resistance has been termed parasitic erosion of horizontal resistance (Robinson, 1976).

Resistance may also decrease with increase in inoculum density. Generally, the polygenic resistance, expressed at intermediate level is more affected by inoculum density; monogenic, vertical resistance is less affected. For example, monogenic vertical resistance to rusts in cereals is hardly affected by inoculum density while slow rusting resistance decreases with increase in inoculum density. An inverse relationship between resistance and inoculum density is especially evident with soil borne pathogens (Bell and Mace, 1981). The effect of inoculum density on resistance is also known to depend on the nature of the propagule of the pathogen.

Effects of environment

Environment may affect the host and the pathogen differentially. Even when the optima for host colonisation by the pathogen and expression of the host resistance for a particular environmental factor are the same the actual level of observed resistance may vary from the maximum to the minimum depending on the particular host pathogen combination (Bell, 1982). Individual environmental factors may also affect disease development through effects on other environmental factors.

Cultural conditions like the composition and pH of the medium, light intensity and temperature may affect the pathogenicity of the pathogen. If effects of environmental factors are not taken into consideration in disease resistance studies misclassifications of resistance are possible (Kulkarni and Chopra, 1982) because of interactions between genotype (host/pathogen) and environment. Effect of various environmental factors on resistance to soil borne pathogens has been extensively and critically reviewed by Bell (1982).

Effect of environment on genetics of resistance

The genetics of resistance to some diseases is strongly influenced by physical or biological environment. Temperature appears to be a major factor in determining dominance status of the genes for resistance. Vanderplank (1978)

cites examples of resistance genes which are dominant at lower temperatures and recessive at higher temperatures. Such genes include Tm I, Tm 2 and Tm 3 of tomato for resistance to tobacco mosaic virus and genes for resistance to bean yellow mosaic virus in peas.

Resistance to *Verticillum* wilt in cotton has been found to be recessive, additive or dominant at 25, 27 and 29° C, respectively (Bell, 1982).

Slow mildewing resistance in wheat has been found to be monogenically inherited under controlled environmental conditions of greenhouse while it is polygenically inherited under uncontrolled environmental field conditions (Ellingboe, 1981).

Genetics of resistance can be confusing when two or more pathogens are involved and the breeder is not aware of this fact. Resistance to *Fusarium* wilt in tomato, is a good example of this situation. Sidhu and Webster (1983) showed that wilt resistance was monogenic when nematode infection was checked and polygenic when scoring was done on material experimentally inoculated with root knot nematodes.

Parasitic epistasis (Sidhu, 1984) that is, effect of one pathogen (or parasite) on the resistance or susceptibility to another, is also a factor which may lead to incorrect conclusion about the number of genes involved in determining resistance to a pathogen. Tomato plants susceptible to *Verticillum* but resistant to *Fusarium* are resistant to both the pathogens when the soil harbours both *Fusarium* and *Verticillum* (Sidhu and Webster 1979). A F_2 population segregating for resistance versus susceptibility to the two pathogens, when inoculated with both the pathogens, would show 3 resistant: 1 susceptible ratio for *Fusarium* but 15 resistant: 1 susceptible ratio for *Verticillum* although resistance to *Verticillum* is controlled by only one gene. Resistance to *Fusarium,* however, breaks down if root-knot nematodes are also present in the soil. If a F_2 population segregating for resistance and susceptibility to both *Fusarium* and nematodes is inoculated with both the pathogens, a 3 resistant: 1 susceptible ratio would be obtained for nematodes while 9 resistant: 7 susceptible ratio would be obtained for *Fusarium* (Sidhu and Webster, 1974). These findings emphasize the importance of eliminating the influence of all pathogens other than the one under study while conducting genetic tests on disease resistance.

Breeding for disease resistance

In breeding programmes, disease resistance is handled like any other trait except that special conditions for pathogen multiplication are required to screen and recognise the resistant plants.

Sources of disease resistance

Resistance may be found in locally adapted or exotic cultivars, or in related species. Locally adapted cultivars are good sources of resistance against diseases which have assumed importance because susceptibility for them was unintentionally introduced while breeding for some other character of economic value. Locally adapted cultivars are also a good source of resistance against diseases of minor or local importance. Resistance of locally adapted cultivars is preferable for two reasons: (1) such resistance may carry a smaller number of undesirable linkages than resistance in exotic material, and (2) resistance in exotic cultivars may be linked with susceptibility to some diseases or pests to which locally adapted cultivars are generally resistant. The use of exotic donor can lead to unintentional incorporation of such susceptibility which may later pose serious problem.

Primary and secondary centres of origin of both the host and the pathogen are considered to be good regions for locating resistance. Resistance genes present under conditions of both high and low disease severity do not seriously affect the fitness of the host (Leonard, 1983).

It is however, not necessary that resistance is found only in centres of origin of the crop (Harlan 1977). Resistance to barley yellow dwarf, for ex-ample, is known only from Ethiopia. Similarly, resistance to neck blotch in barley has been found in accessions from China, Japan, Korea and Ethiopia (Caddel and Wilcoxson 1975; Qualset, 1975) while resistance to soybean mildew has been found in accessions from Assam and Nepal (Harlan, 1977).

Resistance can also be derived from distantly related species and genera. Wheat varieties such as Transfer, Agatha and Compair have, for example, derived resistance, from related species or genera (Knott and Dvorak, 1976). It should be remembered, however, that resistance from alien species or genera may be no more durable than resistance from close relatives. A notable example of such resistance genes from another species which were soon overcome by the pathogen are R-genes for resistance to potato late blight derived from *Solanum demissum*. Resistance may also be obtained through induced mutations (Sawhney *et al.*, 1978) or through tissue culture (Larkin and Scowcroft, 1983).

Breeding procedures

The commonly used procedures of breeding for disease resistance are: (1) backcross breeding, (2) recurrent selection, and (3) interspecific hybridisation.

Backcross breeding

This procedure is usually followed when resistance is simply inherited and an agronomically superior cultivar is available to which the transfer is sought. Polygenically governed resistance can also be transferred by back-crossing if the heritability is moderately high. For polygenically controlled resistance, many more families of backcross progenies and several plants within each family need to be grown in comparison with backcross breeding for monogenic resistance.

Recurrent selection

Such selection is generally followed when resistance is polygenically inherited and the crop is cross-pollinated. There are several examples where rapid improvement in resistance has been obtained through recurrent selection. But the most widely quoted example is that of African maize to rust caused by *Puccinia polysora*. Recently, Heisey and Murphy (1985) increased the percentage of plants resistant to *Phytophthora megasperma* in *Medicago falcata* and *M. caerulea* from 3 and 13 per cent to 78 and 62 per cent respectively, in three cycles of phenotypic recurrent selection for resistance. Robinson (1976; 1980) has advocated recurrent selection, in conjunction with the use of male gametocides, for increasing levels of resistance to diseases in self-pollinated crops. Use of this method has been attempted recently in rice, wheat and barley (Choudhary *et al.*, 1981; Beek, 1983; Sharp, 1983).

Interspecific hybridisation

Transfer of resistance from related species or genera to crop cultivars employs cytogenetical techniques for chromosome engineering. These have been reviewed by Knott and Dvorak (1976); Joshi and Singh (1977); and Driscoll (1983).

Breeding for multiple disease resistance

For a variety to be successful, it must possess resistance to most prevalent major diseases which cannot be effectively controlled by other methods. A preferred method of ensuring stability of yield of crop cultivars is to incorporate, what has been termed as, 'multi-adversity resistance'. Adversities include diseases and pests, unfavourable soil conditions and fluctuations within and between seasons. The effect of multi-adversity resistance on yield stability of a cultivar is illustrated in Fig. 1. The fluctuations in yield of the one time popular rice cultivar IR 8 (released by IRRI in 1966), which carries resistance to one major pest, and moderate resistance to one disease and two soil deficiency conditions, are wider compared to fluctuations in IR–36 and IR–42 which carry resistance to four diseases, three pests and resistance or moderate resistance to at least three soil deficiency problems (Khush, 1984).

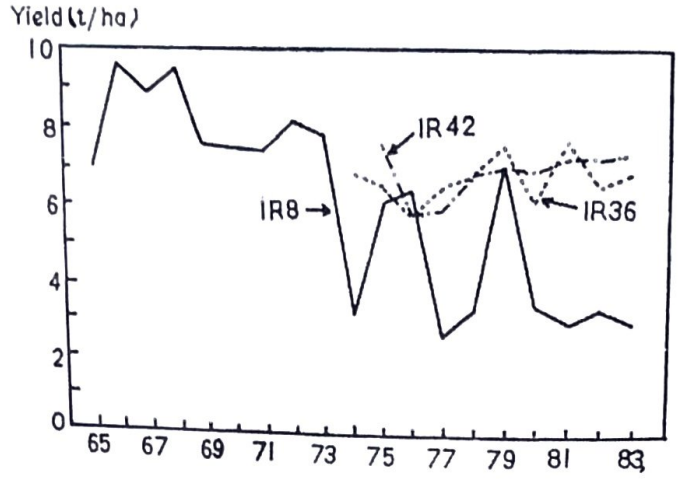

Fig. 1: Yields of IR8, IR36, IR42 in dry season from replicated yield trials at IRRI.

Improvement for multiple traits like resistance to different diseases and pests, may be done either in tandem order (sequential incorporation of resistance) or by applying simultaneous selection for different diseases and pests during breeding. Hanson *et al.* (1972) for example, applied selection in alfalfa for resistance to leaf hopper, leafspot, bacterial wilt and anthracnose in a phased manner.

The sugar cane breeding programmes at Hawaii and the cotton breeding programmes at Texas, are outstanding models of multiple disease resistance programmes. The salient features of the Hawaiian sugar cane breeding programme are utilisation of a broad genotypic base provided by species and species derivates, extensive crossing, intense selection for resistance to major and potential pathogens and pests both local and exotic and localised testing in all environments where the new variety is to be grown (Buddenhagen, 1977). The multi-adversity resistance programme in cotton followed at Texas Agricultural Experiment Station involves testing for germination at low temperature (14°C), followed by screening for resistance to soil pathogens (*Rhizoctonia, Pythium,* and *Thailaviopsis*)in naturally infested soil. The selected seedlings are tested against races 1, 2, 7 and 18 of *Xanthomonas malvacearum.* The progeny of selected plants are distributed to different nurseries where they are exposed to insect pests, nematodes and other diseases. Only those lines that show resistance to all adversities are then advanced in the breeding programme (Bird, 1982).

The population improvement programme advocated by Robinson (1973; 1976; 1980) for improving horizontal resistance in cereals to pathogens such as rusts, using male gametocides, aims at simultaneous improvement of resistance to several important pathogens. This method has been tried in wheat and simultaneous improvement has been obtained for yield, and resistance to leaf rust, stem rust, powdery mildew, *Helminthosporium,* and *Septoria* (Beek, 1983).

The results have convincingly demonstrated the feasibility of the new method of breeding for resistance advocated by Robinson. Maize stocks multiply resistance to a number of diseases have been developed in India (Payak and Sharma, 1979).

Genetic of host-pathogen interaction

Flor (1942) was the first to study the genetics of both resistance in the host and pathogenicity in the pathogen. The host pathogen combination studied was flax and flax rust fungus. The findings of his studies led to the discovery of gene-for-gene relationship between resistance in the host and virulence in the pathogen and to the formulation of gene-for-gene concept which states that for each gene conditioning resistance in the host, there is a corresponding gene conditioning pathogenicity in the pathogen.

Loegering and Powers (1962) pointed out that Flor's analysis could be represented in a general form as four possible interactions among a pair of alleles governing resistance in the host and a corresponding pair of alleles governing virulence in the pathogen. The following form depicting the four possible interactions has come to be known as "quadratic check" (Table 3).

Table 3: The quadratic check resulting from interaction between two alleles at one locus in the host and two alleles at one locus in the pathogen

Pathogen	Host	
	R-	rr
A–	Resistant	Susceptible
aa	Susceptible	Susceptible

A resistant reaction occurs when the host gene for resistance interacts with the corresponding gene for avirulence in the pathogen.

In the quadratic check, only one locus in the host and one in the pathogen is considered. Conformity to disease expression in the quadratic check is not a proof of a gene-for-gene hypothesis. This is because the hypothesis states that for each gene conditioning resistance in the host there is a corresponding gene conditioning virulence in the pathogen. Therefore, a minimum of two resistance genes in the host and two complimentary virulence genes in the pathogen are necessary to demonstrate a gene-for-gene relationship.

Table 4. Interaction between resistance genes at two loci in the host with corresponding virulence genes at two loci in the pathogen

Pathogen	Host	
	R_1–	R_2–
r_1r_1	Susceptible	Resistant
R_2 r_2	Resistant	Susceptible

It is apparent from Table 4 that, in contrast to the quadratic check, the resistance gene in the host is identified by the corresponding gene for virulence in the pathogen. Consequently, susceptibility is specific and resistance is not (Vanderplank, 1984). For example, a potato variety with gene R 1 is susceptible to race (1) or any other race with 1 in its designation such as races (1, 2), (1, 2, 3), (1, 3, 4) etc., but is resistant to all other races lacking the gene for virulence capable of overcoming resistance of gene R 1. This has been the basis of naming races and resistance genes in potato-*Phytophthora* system. Another aspect of the above interaction table is the differential interaction between resistance genes and virulence genes. Therefore, all gene-for-gene controlled diseases involve vertical resistance.

The quadratic check, where resistance occurs in only one of the four combinations of the host and the pathogen genotypes, is the most common pattern observed with biotrophic pathogens. With pathogens that produce host-specific toxins, like *Helminthosporium victoriae, H. maydis, H. turcicum, H. carbonum, Periconia circinata,* an opposite pattern is found (Table 5).

Table 5. The quadratic check observed with pathogens that produce host-specific toxins

Pathogen	Host	
	R–	rr
A– (Produces toxin)	Resistant	Susceptible
aa (does not produce toxin)	Resistant	Resistant

This pattern of quadratic check is also not a proof of gene-for-gene relationship for the reason already stated (that is, the involvement of only one locus in both the host and the pathogen). The point is illustrated in the following using the example of maize—*H, turcicum* system (Table 6).

Table 6. Interaction between resistance genes in maize with races of *Helminthosporium turcicum*

Race	Resistance gene	
	Ht1	Ht 2
1	R	R
2	S	R
3	R	R

The interaction between races 1 and 2 of *H. turcicum* and dominant resistance genes Ht 1 and Ht 2 gives a quadratic check (Table 6) that is found with pathogens producing host-specific toxins. Lim *et al.* (1974) found that virulence in *H. turcicum* on resistance gene Ht 1 was controlled by a single gene and, therefore, they concluded that maize—*H. turcicum* interaction fits the gene-for-gene relationship. Vanderplank (1978) argued that the proof for a gene-for-gene relationship in the above disease can be had only if another race capable of overcoming resistance of gene Ht 2 but not of gene Ht 1 can be found (as race 3 in Table 6) because only then each gene for resistance in the host would have corresponding gene for virulence in the pathogen. Such a race that is, race 3 has been reported by Smith and Kinsey (1980). Race 3 is virulent on resistance gene Ht 2 and on another resistance gene Ht 3 but avirulent on Ht 1 (both races 1 and 2 are avirulent on resistance gene Ht 3). To prove a gene-for-gene relationship in maize—*H. turcicum* system, it now only remains to be shown that virulence in *H. turcicum* on resistance gene Ht 2 is governed by a single gene.

A gene-for-gene relationship has been found for most classes of pathogens, especially where mono- or oligogenic resistance has been used for developing disease resistant cultivars. However, whether polygenic resistance also operates on a gene-for-gene basis with pathogenicity genes is disputed. Evidences of small race-specific effects have been obtained for some host-pathogen interactions (Parlevliet, 1977; 1978; Mortensen and Green, 1978). Parlevliet and Zadoks (1977), using a hypothetical example, argued that with polygenic systems, the effects of individual genes are small and the possible differential interactions are likewise small. Therefore, if the magnitude of experimental error is the same or greater than that of interaction effects, it will be difficult to detect such interactions.

Vanderplank (1968; 1978), and Robinson (1976) assumed that resistance genes involved in horizontal resistance do not operate on a gene-for-gene basis with genes for pathogenicity in the pathogens. Since horizontal resistance is assumed to be governed by polygenes, Vanderplank (1978) used the example of oligogenically controlled horizontal resistance to dispel the belief that durability

of horizontal resistance is due to polygenic control of such resistance. It has been argued by some that durability of horizontal resistance arises from polygenic inheritance of pathogenicity in the pathogen (Vanderplank 1963; Eenik, 1976) and that it is immaterial whether the resistance itself is inherited oligo or polygenically. The basis for this argument is derived from the genetic theory of selection. For polygenically inherited traits, the intermediates rather than the extremes in the population are best suited to survive. The durability of horizontal resistance, therefore, arises from stability of races of the pathogen. Fleming and Persons (1982) attributed durability of horizontal resistance to "phenotypic damping" and "slow response to selection". Given the polygenically inherited (and phenotypically normally distributed) horizontal resistance in the host and aggressiveness in the pathogen, a change in the mean or variance of the phenotypic distribution of either interacting population transmits a relatively small change in the corresponding parameters of disease reaction. For a polygenically determined trait, substitutions for many genes are required to produce a substantial change in the phenotype of the trait. Such a change takes a long time to materialise especially if a specific substitution at a particular locus is necessary before a subsequent substitution at another locus becomes adaptive. Thus "slow response selection" and "phenotypic damping" account for durability of polygenically controlled horizontal resistance. However, since all horizontal resistance is not polygenically controlled, Robinson (1976) has argued that horizontal resistance operates through mechanisms not within the reach of microevolutionary changes of the pathogen.

Epidemics and agriculture

It is widely accepted that diseases, even when present in natural plant communities, do not reach devastating proportions of an epidemic. The hosts and the pathogens co-exist without endangering each other's existence. This stability of the host-pathosystems in the natural ecosystems has been attributed to the diversities in the host, the pathogen and the environment. The components of diversity within the host in a natural ecosystem, include diversity in immunity, vertical resistance, horizontal resistance, physiological age, number of species, horizontal and vertical disposition of host plants and so on. Differences in virulence, aggressiveness, ability to survive unfavourable environments, dependence on insects or other agents for production, release, dissemination and deposition of inoculum etc. contribute to the diversity in the pathogen component of the ecosystem. The diversity in the environmental component is provided by variations in climatic, edaphic and biotic factors including micorrhizae, saprophytes, hyperparasites and succession of organisms (Schmidt, 1978). The complex and constantly changing interactions that occur between various components of the host, the pathogen and the environment ensure that

neither the most resistant components of the host nor the most virulent components of the pathogen dominate the eco-system. The importance of such interactions for maintaining a stable host-pathogen relationship was experimentally demonstrated, recently, by Heather and Chandrashekar (1982). They studied the interactions between poplar cultivars and races of rust pathogen *Malampsora larci-populina* by varying light intensities, temperatures and species of a fungal hyperparasite, *Cladosporium*. It was found that these factors (temperature, light intensity and the species of hyperparasite) had a more dominant role in determining disease levels than either host cultivars or pathogenic races. A still more interesting finding was that interactions involving climatic or biotic components of the environment were of greater magnitude than race-cultivar interactions.

Primitive agricultures retained most, if not all, of the genetic diversity and spatial complexity of the natural ecosystem. The farms were small and far apart and each farmer grew crops of his own choice to meet personal family requirement. Mostly mixed cropping was followed. Crop rotations or closed seasons were thus ensured. Natural selection in genetically heterogenous crop cultivars ensured moderate levels of resistance to major pathogens with the result that diseases, although always present, rarely reached epidemic proportions.

Some disease epidemics did occur before conscious efforts were made to control diseases through plant breeding. These epidemics resulted largely from introduction of pathogens to a new region, from the movement of the host to a new region or from movement of both the host and the pathogen separately to a new region. The 1840 epidemics of late blight of potato in Europe: Dutch elm disease epidemic in the U.S.A. and the U.K.; chestnut blight and pine blister rust in the U.S.A. and maize rust in West Africa are some of the examples. The hosts in such situations had evolved in the absence of the pathogen and therefore, lacked previous adaptation. It is also known, however, that erosion of resistance occurs in the absence of the pathogen.

Modern agriculture brought about so many changes in cultivation practices that the natural ecosystem was drastically changed. It considerably narrowed the genetic base of crops. Man today depends on only 15 species of plants for his food compared with about 3,000 species of plants which he used as food during history (Mangelsdorf, 1966). The genetic diversity, even within these few species, has been reduced to a few cultivars largely because farmers adopt the newest and the highest yielding cultivar rapidly and leave little time for breeders to establish a broad spectrum of varieties. Thus, large areas of land are sown only to a few superior cultivars. The new agricultural technology also brings about other changes such as crop cultivation at high plant densities with high levels of fertiliser application. Fields are now sown at more or less the same time.

Development of photoinsensitive varieties has enabled farmers to grow the same crop throughout the year. All these conditions are well known to be highly conducive to disease development. Crop protection has become an integral component of intensive agriculture and in the Indian situation, has depended heavily on breeding of disease resistant varieties. The degree of success in breeding resistant varieties, however, has varied both between crop and between diseases affecting a crop. A majority of the epidemics which have attracted the attention of plant breeders and pathologists recently, have resulted either directly or indirectly from plant breeding activity.

Epidemics of Victoria blight of oats and southern leaf blight of maize in the U.S.A.; of pearl millet downy mildew, of bacterial blight and tungro diseases of paddy and of Karnal bunt of wheat in India are consequences of inadvertent breeding for susceptibility. Generally, screening for resistance to minor or economically unimportant diseases is not carried out. When genetic materials of unknown susceptibility to some diseases are used in the breeding programmes, inadvertent incorporation of susceptibility can occur. If the resultant varieties come to occupy large areas, they pave the way for large scale epidemics. Buddenhagen (1977) has suggested ways of circumventing release of varieties with unknown susceptibility. He recommended that before a new variety is released, the area where it will be grown should be carefully screened for major and minor diseases and pests existing on varieties under cultivation and an assessment should be made of their relationship with the agricultural technology. If the existing agricultural technology in the area is primitive and the new variety is to be recommended for cultivation along with a new technology, then the impact of new technology should be studied first on the old varieties to determine if the changed technology causes a pathogen shift.

Breeding for vertical resistance, and the consequent "boom and bust" cycles, are striking examples of direct consequences of the incorporated genes for resistance. Individual vertical resistance genes are extracted from natural populations of the host plants (where they provided population resistance) and are used for developing disease resistant varieties. When such varieties came to occupy large areas, a tremendous selection pressure is exerted on the pathogen and races matching the resistance in such cultivars are selected. These races eventually render the varieties susceptible. It is now accepted that breeding for vertical resistance has not provided durable disease control against air borne fungal pathogens of self-pollinated cereals, potato, tobacco, coffee, rubber and many other crops (Simmonds, 1979). This failure of vertical resistance is a consequence of lack of diversity in resistance genes used in the development of disease resistant cultivars. Parlevliet (1981) has opined that the most common strategy employed in breeding a crop for disease resistance has been no strategy

at all. As a result most resistant cultivars developed for a region carry the same gene or genes because they represent the best source of resistance. The situation equally holds good for Indian wheat breeding programme (Nagarajan *et al.*, 1984). The farmer's desire to cultivate only the best variety has also contributed to this problem. Since vertical resistance has evolved in essentially heterogeneous host populations, diversity of resistance genes is essential for effectiveness of VR genes. The different strategies of using vertical resistance genes, such as temporal, geographical or spatial development of genes, use of multilines, cultivar mixtures or multigenic varieties, can be effective only when diversity of resistance genes is ensured. However, deployment of different genes in time and space poses operational problems. No compelling evidence is available which shows that multilines would stabilise pathogen populations and provide lasting resistance (exception being oat multilines in the U.S.A). Although some multigenic cultivars have been more durable than others, prediction, as to which combinations of resistance genes would be more durable than others is not possible at present. Vanderplank's (1978; 1982) hypothesis of dissociation between virulence genes as the basis of durable vertical resistance of some of the multigenic host cultivars has also been questioned (Wolfe and Knott 1982; Knott, 1984). Therefore, the use of vertical resistance in breeding for disease resistance (except in case of less mobile pathogens) has come under severe criticism during recent years and plant breeders are being called upon to concentrate on horizontal resistance (Robinson, 1976; Simmonds, 1979; Duvick, 1984). Although breeding for horizontal resistance is more laborious, it is encouraging that for all practical purposes, only modest levels of horizontal resistance may be all that is required. Also, population improvement programmes, using male gametocides or sterility genes in self-pollinated crops have demonstrated that simultaneous improvement could be obtained for several traits, including yield and resistance to different diseases (Beek 1983). Such comprehensive breeding programmes also take care of minor and locally important diseases and pests and help in avoiding, inadvertent incorporation of susceptibility to diseases and pests.

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7

Breeding for Protein Quantity and Quality

H.C. Bansal and S.L. Mehta

The immediate and long-term objective of modern plant breeding in all countries irrespective of the state of their advancement is increasing the availability of food to meet the calorie-protein requirement of their people. In the past, breeders have concentrated their efforts on increasing the yield of the important crop species. Better adaptation to local environmental conditions, high production and better resistance to the major pests and diseases have been among the priority goals of genetic plant improvement. Yielding ability and stability undoubtedly deserve high priority but enough attention also needs to be paid to improving nutritional characteristics of the plant produce for their maximum utilisation. Awareness of nutritional requirements and the optimal characteristics of crop products to be utilised as food and feed, therefore, becomes an important parameter of breeding effort.

The main source of food in the developing world is cereals which provides 45 to 85 per cent of the total calories and 50 to 80 per cent of the total protein requirements. The consumption of animal proteins, which are nutritionally well balanced, is low and costly and beyond the reach of the low income groups. On the other hand, plant proteins are cheaper to produce and easier to store and transport. All these factors go in favour of increasing the quantity and quality of cereal proteins in order to improve the dietary and nutritional status of the masses in the developing world.

Proteins are made up of about twenty different amino acids. For man, eight of these amino acids, valine, methionine, leucine, isoleucine, threonine, phenylalanine, lysine and tryptophan, are essential (EAA) for constituting a well balanced diet. In impioving the grain quality of cereals consideration should, therefore, be given apart from the content of carbohydrates, fats, minerals and vitamins in

the grains, to improving the protein content as also the amino acid composition of the protein.

From nutritional point of view, the quality of protein is as important as its quantity. FAO/WHO (1973) have given the essential amino acid composition of a reference protein with which all food proteins are compared (Table 1). On this basis, cereals are generally deficient in lysine, tryptophan and/or threonine while pulses are deficient in sulphur containing amino acids. Consumption of pulses and cereals in the ratio of 1 : 3 meets the nutritional needs ideally because deficiency of sulphur amino acids in pulses is supplemented by their excess in cereals and the lysine deficiency of cereals is made good by its excess in pulses.

However, due to lower availability of pulses and due to their high cost, people cannot afford the consumption of pulses in the proportion recommended by nutritionists. Till such time as the yield of pulses is substantially increased to bring down their prices, cereals will continue to be the major dietary source of proteins. For removing protein malnutrition, improving the nutritional quality of cereals assumes priority.

The global problem of malnutrition requires that the earth's land resources are utilised more efficiently. One way of doing this is to increase the crop yields, another is to develop varieties with higher nutritional quality without adversely affecting their yield. Therefore, selection criteria in addition to productivity must also include the following considerations:

1. Productivity = Yield (kg/ha) × Nutritive value × Technological value
2. Nutritive value = Percentage of protein/calories or utilisable protein
3. Technological value = All functional characteristics as percentage of the standard variety.

Need for quality improvement

It has been well documented that protein quality of the diet has tremendous effect on the overall growth and brain development. In addition, protein malnutrition leads to several diseases like kwashiorkor and marasmus. When the protein intake is adequate but the calorie intake is too low, general protein-energy malnutrition is also observed because ingested food proteins are metabolised for energy production instead of being used for building up body proteins. Some food crops besides lacking in adequate amounts of nutrients such as essential amino acids, minerals and vitamins, contain antinutritional factors (trypsin inhibitors, phytohaemagglutinins, lathyrogens, cyanogens,

Table 1: Amino acid requirements of infants, pre-school children and adults (FAO/WHO, 1973)

Amino acid	Infants		Pre-school children		Adults	
	mg/g protein	mg/1.85 g ideal protein	mg/g protein*	mg/0.9g ideal protein	mg/g protein	mg/0.55 g ideal protein
Lysine	52	96	55	50	22	12.1
Threonine	44	81	40	36	13	7.2
Tryptophan	8.5	15.7	10	9	6.5	3.6
Total sulphur amino acid	29	54	35	32	24	13.2
Leucine	80	148	70	63	25	13.8
Isoleucine	35	65	40	36	18	9.9
Valine	47	87	50	45	18	9.9
Total aromatic amino acids	63	116	60	54	25	13.8

Ideal protein requirements per kg body weight per day:

Infants	—	1.85g
Pre-school children	—	0.9 g
Adults	—	0.55 g

*Denotes FAO reference protein composition.

glycosides, tannins, etc.). These further reduce the bioavailability of the limiting nutrients or induce other toxic effects. Hence, the main nutritional goals for plant breeders are the following:

1) Increased protein content
2) Improved amino acid pattern
3) Increased protein digestibility
4) Reduced content of antinutritional factors affecting food utilisation.

Breeding for protein quantity and quality

a) Cereals

Concerted efforts on the genetic upgradation of the nutritional quality of cereal protein were initiated following the discoveries of the association of Opaque-2 (Mertz *et al.*, 1964) and Floury-2 (Nelson *et al.* 1965) genes in maize with increased lysine. The lead given by the Purdue group was soon followed by an exclusive search for nutritionally superior genotypes in the world cereal germplasm collections and from induced mutants. This resulted in the identifications of Opaque-7 (McWhirter, 1971) and brittle genes (Misra *et al.*, 1972) in maize, Hily and Hiproly (Munck *et al.*, 1970), Riso 1508 (Ingversen *et al.*, 1973; Doll *et al.* 1974), Notch-1 and Notch-2 (Bansal, 1970; Balaravi *et al.*, 1976) genes conferring higher lysine and/or high protein in barley and high lysine genes IS 11758, IS 11658 and Opaque P 721 (Singh and Axtell, 1973; Axtell 1976) in sorghum (For a summary please see Table 2).

One of the pathways of improving protein content in cereals is to break the negative correlation between protein content and grain yield. Grain filling has been the major constraint in the high lysine genotypes so far identified in cereals like maize, barley and sorghum. By rigorous breeding and selection, possibilities do exist of recovering plump grains combined with high protein and/or high lysine as shown by Jain *et al.* (1976) in wheat and Bansal *et al.* (1980) in barley.

Characterisation of protein mutants

All over the world attempts are being made to breed for improved protein in cereal grains. The first point is how to define a genotype with improved protein? When one measures the percentage of protein in a sample, it may be greater simply because starch is reduced due to adverse effects on one of the many biochemical processes controlling grain filling (Scholz, 1976). Protein percentage should be considered only in combination with 1000-kernel weight and other yield components to eliminate undesirable characters such as shrunken grains. A protein mutant may be defined as one which is characterised by higher protein

Table 2: Examples of the genes affecting protein quality in cereals

Crop	Mutant genotype	Spontaneous/ Induced	Quality attributes	Reference
Maize	Opaque-2	Spontaneous	High lysine and tryptophan	Mertz *et al.*, 1964
	Floury-2	„	High lysine and sulphur containing amino acids	Nelson *et al.*, 1965
	Opaque-7	„	High lysine and tryptophan	McWhirter 1971
	Brittle-2	„	Modified starch, high lysine, tryptophan	Misra *et al.*, 1972
Barley	Hiproly	„	High protein-high lysine	Munck *et al.*, 1970
	Notch-1\ Notch-2	EMS	-do-	Bansal, l970
	Riso 1508	El	High lysine	Balaravi *et al*, 1976 Ingversen *et al.*, 1973 Doll *et al.*, 1974
Sorghum	IS 11167 IS 11758	Spontaneous	High lysine (hl)	Singh and Axtell, 1973
	P-721 Opaque	DES	High lysine	Axtell 1976

quantity per seed and/or per plant and/or per unit area and/or a changed amino acid composition. For the guidance of the breeder working with quantitative protein improvement, it is essential to minimise the environmental variability (*e.g.* growing the breeding population under uniform conditions and to confirm results through replication in locations and seasons).

For the qualitative protein improvement, consideration should be given to the high lysine mutants. Except for hiproly barley (Munck *et al.*, 1970), other high lysine genotypes in maize (Mehta *et al.*, 1973), barley (Shewry *et al.*, 1979) and sorghum (Singh and Axtell, 1973) have been found to have impaired prolamine, the alcohol soluble fraction, which is particularly rich in the dicarboxylic amino acid, glutamic acid and proline but is especially poor in the basic amino acids, including lysine. Thus, if one can reduce the relative proportion of prolamine in the grain and enhance the other and better balanced proteins, higher lysine content is achievable.

The seed storage proteins in cereals have been classified on the basis of their solubility into: (i) albumin, the water soluble; (ii) globulin, the salt soluble; (iii) prolamine, the alcohol soluble, and (iv) glutelin, the alkali soluble. The proportion of these major storage proteins in cereals is shown in Table 3 and their amino acid composition is given in Table 4. It is observed that cereals are rich in prolamine which accounts for 40-50 per cent of the total seed protein in maize, barley and sorghum. The poor nutritional quality of the cereal grains, therefore, is mainly on account of predominance of prolamine fraction which is extremely deficient in lysine and tryptophan. The high proportion of leucine to isoleucine in this fraction has also been shown to lead to pellagra in population subsisting mainly on maize and sorghum. The improvement in nutritional quality of high lysine genotypes is mainly due to a decrease in the prolamine fraction (Table 3). From the nutritional point of view, albumin, globulin and glutelin have a balanced essential amino acid (EAA) composition. In Opaque-2 maize, the high concentration of lysine and tryptophan as compared to normal maize is a direct consequence of depressed zein synthesis (Mehta *et al.*, 1973). Studies carried out in developing kernels indicate that albumin decreases while prolamine fraction increases during later stages of grain development. The prolamine fraction of various cereals share similarities of amino acid composition but vary in complexity and in molecular weight of their sub-units.

In maize, two major polypeptides with molecular weights of 19000 and 22,000 daltons representing about 80-90 per cent of zein have been detected. In addition, two polypeptides with MW of 10,000 and 15,000 daltons are also present. In high lysine Opaque-2 mutants, the reduction in total zein is mainly accounted for by a substantial reduction in the proportion of the 22000 MW polypeptides.

The extent of reduction, however, varies in different inbred lines of maize. Analysis of zein of normal and Opaque-2 maize by isoelectric focussing (IEF) between pH 5 and 9 showed heterogeneous distribution of polypeptides. The 22 and 19 kd polypeptides are coded by separate but similarly sized mRNAs, approximately 1100 nucleotide length (Larkins and Hurkman, 1978; Wienand and Feix, 1978).

Table 3. Protein fractionation in cereal grains

Cereal	Per cent of extracted protein			
	Albumin	Globulin	Prolamine	Glutelin
Wheat	11.5	16.0	45.5	27.0
Milled rice	6.4	8.8	3.0	81.6
Maize—Opaque-2 endosperm	28.8	13.1	14.5	43.7
Normal	4.0	2.0	55.2	39.0
Barley—NP113Notch-2	23.3	12.6	17.0	47.1
	27.8	11.8	14.0	46.4
Sorghum—Normal High lysine	6.1	16.9	37.9	39.1
	27.9*		25.1	47.0
Pearl millet	15.1	6.6	36.9	41.4

*Albumin + Globulin

In barley, SDS-PAGE of hordein reveals several sub-units varying in MW from 20,000 to 86,000. Arbitrarily, hordein has been divided into three groups and designated A, B and C (Koie *et al.*, 1970). Group A polypeptides have MWs of 20,000 or less. They are soluble in 55 per cent isopropanol, their amino acid composition is similar to that of globulin but they have a high lysine content. The major hordeins occur in Group B and have MWs 30,000-50,000. In normal variety Bomi of barley, proteins with MWs of 30,000, 43,000 and 51,000 are the most prominent. Group C hordeins have MWs about 60,000-80,000 and although they have an amino acid composition typical of prolamines, they represent only a small proportion of the total protein.

SDS poly-acrylamide gel electrophoretic analysis of hordein from mature NP 113 and Notch-2 barley endosperms showed nine polypeptides in NP 113 and 7 in Notch-2. The polypeptides present in Notch-2 were also present in NP 113. The two polypeptides with molecular weights 65 kd and 30 kd were observed only in NP 113 hordein (Bhattacharya *et al.*, 1986). In Riso 1508 mutant, in addition to a quantitative reduction in prolamine content, the proteins are also altered qualitatively.

b) Legumes

The legumes are generally rich in proteins, content of which in major legumes varying from 18 to 28 per cent. This is double that of cereals and slightly higher than that of meat, fish and eggs. Soybean is exceptionally high in protein content (43 per cent protein). The major storage protein in the legumes is the globulin protein, and accounts for about 80 per cent of the total seed protein. Globulin has been further fractionated into alpha, beta and gamma-globulin. In *Cicer arietinum* α-globulin accounts for over 80 per cent of the total globulin followed by β and γ-globulins (Srivastava *et al.*, 1977). The deficiency of sulphur amino acids in a-globulins detracts the nutritional quality of pulses. In *Pisum sativum* and *Vicia faba,* globulin has been fractionated into two major components: legumin and vicilin. For fractionation of salt soluble globulin into legumin and vicilin, Osborne and Campbell (1898) made use of the fact that: (i) legumin was less soluble than vicilin in dilute salt solutions, and (ii) legumin solutions were not coagulable, while vicilin coagulates at 95°C. Vicilin and legumin have been isolated and purified from a number of legumes *viz. Pisum sativum, Vicia faba, Phaseolus vulgaris* (Derbyshire *et al.*, 1976). On ultracentrifugation, vicilin and legumin sediment as single components with S_{20W}, 6.5-8.1 and 12.6 respectively. The relative amounts of these two storage proteins vary considerably between species. For example, *Phaseolus vulgaris* contains very little, if any, legumin (Derbyshire and Boulter 1976) whereas *Vicia faba* contains a substantial excess of legumin over vicilin (Puchel *et al.*, 1979). The amino acid composition of pea protein shown in Table 5 indicates deficiency of sulphur amino acids when compared to FAO/WHO reference protein.

Table 4: Essential amino acid composition of different protein fractions (g amino acid/100 g protein) from sorghum

Essential amino acid	Albumin	Globulin	Prolamin	Glutelin
Isoleucine	4.48	3.31	3.68	4.42
Leucine	6.92	5.42	16.34	8.94
Lysine	4.86	3.92	0.24	3.68
Methionine	2.82	2.34	0.88	1.22
Phenylalanine	2.94	4.82	5.14	4.01
Threonine	4.18	4.32	1.86	1.92
Tryptophan	2.04	2.27	0.24	1.28
Valine	10.16	6.42	5.14	6.52

Table 5. Amino acid compositions of pea proteins (Mole %)

	Total pea protein	Albumin fraction	Globulin fraction	Legumin	Vicilin
Asp	13.36	11.4	12.0	13.07	18.87
Thr	4.22	5.2	3.4	3.08	2.69
Ser	6.12	5.2	5.4	5.77	7.67
Glu	17.23	15.6	19.5	19.47	19.18
Pro	4.99	4.6	4.4	5.50	1.99
Gly	7.62	4.9	4.0	6.89	4.85
Ala	6.59	5.8	3.8	6.01	4.13
Cys	1.17	2.3	0.7	1.21	0.00
Val	5.19	5.1	4.4	4.97	4.84
Met	0.72	1.4	0.6	0.66	0.00
Ile	3.97	4.2	4.2	3.95	5.11
Leu	7.68	6.4	8.5	7.60	9.83
Tyr	2.15	4.4	3.5	3.64	1.91
Phe	3.63	5.0	5.4	2.30	4.67
Lys	6.71	8.7	7.4	4.24	8.10
His	1.86	2.9	2.9	1.75	1.61
Arg	6.80	6.8	9.3	9.99	4.57

Source: Gatehouse *et al.*, 1984.

The Protein Efficiency Ratio (PER), *i.e.*, the gain in weight of the animal per unit weight of protein consumed and the biological value (BV), *i.e.*, the percentage of nitrogen retained by the animal from out of the nitrogen absorbed from the diet, have been determined (Aykroyd *et al.*, 1966) for different legumes. PER, for legume proteins vary between 0.5 and 1.5. For soybean the values range between 1.5 and 2.5. On this scale, most of the cereal proteins range from 1 to 2 and meat and egg proteins from 2.5 to 3.5. The biological value of legume proteins vary from 50 to 70 per cent. The PER values obtained in food mixture comprising pulses and cereals were found to be higher than those of either pulses or cereals when used alone.

Another consideration in assessing the value of food protein is its digestibility. The digestibility values for important pulses range from 70 to 90 per cent.

Most legumes, with the exception of soybean, contain little fat, but are considerably richer in calcium and iron compared to cereals. Typical legume intake provides from 2 to 8 mg of iron daily which represents a major fraction of daily requirements.

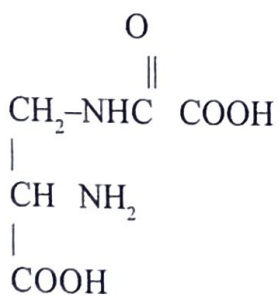
Legumes contain about 60 per cent carbohydrate (mainly starch) which is generally well absorbed and utilised. Soybean, however, contains a considerable amount of carbohydrates such as galactans, pentoses, and hemicelluloses which are poorly utilised.

Most of the legumes contain only small amounts of carotene (Provit A). Values vary from 60 to 700 International Units of Vitamin A per 100 g. The thiamine content of legumes, as a group, is approximately equivalent to or slightly exceeds that of whole cereals. Values range between 0.3 and 0.8 mg per 100 g, with an average of 0.4 to 0.5 mg. Riboflavin content varies from 0.2-0.8 mg/l00g protein. Legumes are, however, fairly good source of niacin, containing on an average 2 mg per 100 g. In addition, the legumes contain biotin, folic acid, inositol, pantothenic acid and pyridoxine.

Toxic substances in legumes

A wide variety of toxic and potentially toxic substances are found among the pulses. These include: protease inhibitors, haemagglutinins, lathyrogens, glycosides, goitrogens, cyanogens, metal binding factors and antivitamin factors. Despite this formidable list pulses have been used for food for thousands of years. Fortunately cooking destroys many of the toxicants present in pulses. Others are present in so small concentration that they are not deleterious in a mixed diet. Nevertheless, there is a possibility that the prolonged consumption of a particular improperly processed legume may bring to surface toxic effects that otherwise would not be apparent. Soaking and boiling destroys the trypsin inhibitors and other toxic materials like haemagglutinins (which destroy haemoglobin). Consumption of uncooked broad beans causes a disease known as flavism characterised by hemolytic anaemia, haemoglobinusia and jaundice. Similarly, gossypol present in cotton seed is toxic. Methods of removing gossypol have, however, been developed.

Lathyrism is the disease associated with consumption of *Lathyrus sativus,* popularly known as kesri dal. In lathyrism, paralysis of the lower limbs occurs. It also affects the central nervous system. The toxic principle has been identified as β-N-oxalyl—α, β, diaminopropionic acid (BOAA).



The amino acid composition of *L. sativus* proteins indicates that they are rich in lysine and are, therefore, potentially useful as lysine supplement if the toxic material can be removed. The detoxification procedure involves steeping the

dehusked seeds overnight followed by steaming for 30 minutes. Alternatively, roasting at 150°C for 70 minutes also detoxifies.

Another approach is breeding cultivars of *Lathyrus* which are genetically deficient in the neurotoxin. In extensive screening of different species and induced mutants of *Lathyrus,* a few accessions *e.g.*, (P-24) have been found to have lower levels of BOAA. Breeding for low BOAA content has brought its content down to 0.03-0.05 per cent, against 0.55 per cent in market samples.

In addition, many legumes contain glycoside from which HCN may be released by enzymatic hydrolysis. Soybean and peanuts produce goiterogenic effects *i.e.*, enlargement of the thyroid gland in animals. For soybean, the causative agent has been shown to be a low molecular weight oligopeptide. In groundnut, it is a phenolic glycoside which resides in the skin. In Brassicae, a thioglycoside called sinigrin, is hydrolysed to produce allylisothiocynate which is the actual goitrogenic agent. These agents are destroyed by cooking or heat treatment.

The per capita availability of pulses in India is only about 40 g/day as against the FAO/WHO recommended intake, for balanced diet, of 104 g/capita/day. Even for making available 60 g/capita/day, the production of pulses has to be increased from the present level of about 12 million tonnes in the country to about 20.5 million tonnes by the end of 1990. As against the present production of 11.17 million tonnes, the planning commission has fixed a target of pulses production at 14.5 million tonnes for 1984-85.

In recent years, there has been an improvement in protein supply, but this increase has largely been due to increased consumption of cereals. In populations which are predominantly vegetarian or depend on vegetable products because of socio-economic consideration, a nutritional imbalance results from predominance of cereals and paucity of pulses in their diet. Since in India, protein derived from animal products represents about ten per cent of the total protein, it is important to increase pulse production by developing high yielding varieties by incorporating disease resistance and by making their cultivation remunerative to farmers. While these efforts will continue another approach offering bright prospects is the exploitation of potential of minor seed legumes. There are several legumes notably, rice bean and winged bean which show great promise. Both are rich sources of protein quality at par with commonly grown pulses (Singh *et al.*, 1985). Protein content of such sources varies from 14 to 24per cent.

Oilseed protein

Several oilseed crops are at present mainly used for oil extraction and for animal feed but they are important sources of supplementing protein for human

consumption. The meal left after oil extraction is very rich in protein; the content varies from 30 to 60 per cent. The amino acid composition of protein from oilseed meal shown in Table 6 indicates its suitability in complementing deficiency in cereals. However, meals of different oilseeds have different nutritional problems, especially the presence of anti-nutritional factors and of foul-smelling substances. Much research efforts have been directed to removing or destroying the toxic constituents of oilseed meals. In the case of soybean, the meal is heat processed to destroy trypsin inhibitors. The other major advance has been to isolate and prepare protein rich concentrates from different oilseed meals. However, the products so manufactured are expensive and beyond the reach of the common man. Therefore, future research efforts will have to be directed at reducing their cost. Attention will also have to be paid in removing gossypol from cotton seed meal, glucosinolates from rape seed meal and aflatoxin from groundnut seed meal.

Table 6: Amino acid patterns of oilseed meals (g/16 g N or % protein)

Amino acid	Soybean	Cotton seed	Peanut	Secame
Arginine	7.5	11.0	10.3	11.9
Histidine	2.5	2.7	2.2	2.2
Isoleucine	5.5	4.0	4.3	4.3
Leucine	7.7	6.2	6.7	6.9
Lysine	6.2	4.2	3.5	2.8
Methionine	1.4	1.5	1.0	2.6
Phenylalanine	4.9	5.2	5.0	4.7
Threonine	4.0	3.5	3.0	3.6
Tryptophan	1.7	1.6	1.2	1.9
Valine	5.4	5.0	4.8	5.1

Source: Lyman *et al.*, 1956.

Breeding strategies

Protein content in cereal grains has been shown to be negatively associated with grain yields, but efforts are being made in several laboratories to combine high yields and increased protein content and quality. Three main plant breeding prerequisites need to be fulfilled for success.

1) Genetic variation should either be present or be created if absent or inadequate.
2) An efficient selection technique should be available which can be effectively applied.
3) The breeding goals should be clearly defined and the material suitably evaluated by employing correct selection procedure for identifying superior recombinants.

Plant breeding essentially consists of three phases: generation of genetic variability, selection of useful genotypes and comparative tests to demonstrate the superiority of the selected genotypes. Breeding for improved nutritional quality is not different from breeding for any other requirement. For combining high protein content and quality with high grain yields, standard procedures of hybridisation and handling the segregating material are followed. The major difference is that analytical techniques are used to mass screen for the desired (*e.g.*, protein, lysine) trait.

The work on wheat, barley, rice and maize has not led to commercial release of a variety bred specifically for quality but attempts at broadening genetic base have been successful. In the following, the status on the major crops worked on is summarised.

Wheat

The initial and the most extensively used genetic source of high protein in wheat was Atlas 66. Also, most is known about the genetic basis for protein in this genotype (Johnson *et al.*, 1968; 1970). Naphal, an introduction from India, has also been widely used because of its elevated content of lysine in addition to high grain protein. Crosses between Naphal and Atlas have yielded selections higher in protein than either Atlas 66 or Naphal (Johnson *et al.*, 1973). These selections, however, have small seed size, are low in productivity and are agronomically poor. Hybridisation of these selections with more productive varieties and high protein genotypes like Rageni has given more productive, high protein lines with more acceptable agronomic traits. Data on crosses of Naphal with other wheats have revealed that protein content is controlled by genes with additive effects (Johnson *et al.*, 1979).

Pusa 5-3, a semi-dwarf, large seeded (average 44 g per 1000 grains) strain with 15 per cent protein content was evolved by intensive selection for high grain weight and high protein content in the early segregating generations of a cross HW 171 × C.I. 11721 (Singhal and Jain, 1981). However, even with its relatively high grain yield, Pusa 5-3 cannot compete for productivity with the best currently released wheat varieties in India. The advanced breeding lines for high protein trait are, therefore, being crossed to newly developed varieties to maintain a continuous improvement in both productivity and nutritional quality. A high protein wheat variety CI 17389 (NE 701132) has been recently released by the University of Nebraska. It combines high yield with high protein content, and indicates the scope for simultaneous selection for yield and quality.

The improvement of the amino acid composition of the protein has proved a complex problem in wheat. Work on lines derived from crosses involving Naphal, Atlas and CI 13449 has not provided clear evidence of the possibility of tackling

amino acid composition by breeding. Also, genes with large effects on lysine level have not been found in wheat.

Barley

In barley, breeding for protein quality has been more successful. This is due to the availability of a number of high lysine genes of diverse origin (Table 2). Genetical, physiological and biochemical work has facilitated the utilisation of these genes in breeding programmes. The high lysine trait in all barley mutants (spontaneous or induced) is controlled by single recessive genes (Munck *et al.*, 1970). The high lysine genes of Hiproly, Notch-2 and Riso 1508 have been shown to be non-allelic (Munck, 1972; Doll *et al.*, 1974; Bansal and Bhaskaran 1973). In high lysine mutants the endosperm is generally noticeably shrunken on the dorsal side near the scutellum (Munck *et al.* 1969; Doll *et al.* 1974; Bansal 1970; Ullrich and Eslick, 1978). For exploiting the full value of high protein and/or high lysine genotypes, the problem of adverse association between endosperm development and protein quality has to be overcome. Breeding strategies for achieving this in barley included crosses between mutants and normal, limited backcrossing (one to three backcrosses) with the normals as recurrent parents and intercrossing between derived (high lysine/protein × normal) lines. Plants visually selected in the F_2, F_3, F_4 and F_5 generations for overall agronomic promise were screened for grain protein and lysine contents. To examplify a backcross programme, the schedule of which is given in Fig. 1, was followed with Hiproly. Sv 73608, a high lysine strain, was derived from Hiproly following five backcrosses to the variety Mona. In this strain the seeds are small but well filled.

It has been reported that in Sweden lines derived from crosses involving Hiproly almost equal the yield of controls under favourable conditions but are more sensitive to hot dry conditions in spring (Persson and Karlsson, 1977). Rhodes and Jenkins (1976) reported that lines derived from crosses involving Riso 1508 produced yields at about the level of the controls. Yields of the most productive high lysine lines derived from crosses of Hiproly and Riso 1508 with adapted Australian cultivars gave yields approaching variety clipper but were 25 per cent less productive than that of the best barley variety (Oram and Doll, 1981).

Continued selfing in the progenies of barley crosses involving high protein and/or high lysine genotypes (Hiproly, Notch-1, Notch-2 and Bl) and the agronomically superior strains (Dwarf mutants BM 18-A and BM 21 and the cultivar Jyoti), leads to marginally better combinations of grain yield and protein quality. For further simultaneous advancement in grain yield and protein quality, recourse to full-sib mating may help in breaking tight undesirable linkages. The progenies of the cross are intermated at random (instead of selfing) and the

generations of the intercrossed population are screened for high yield and high protein and lysine contents. When this approach was combined with selection of plants with performance above the population mean, the effects of negative correlations between 1000 grain weight and high protein-high lysine content became less pronounced (Bansal *et al.*, 1980).

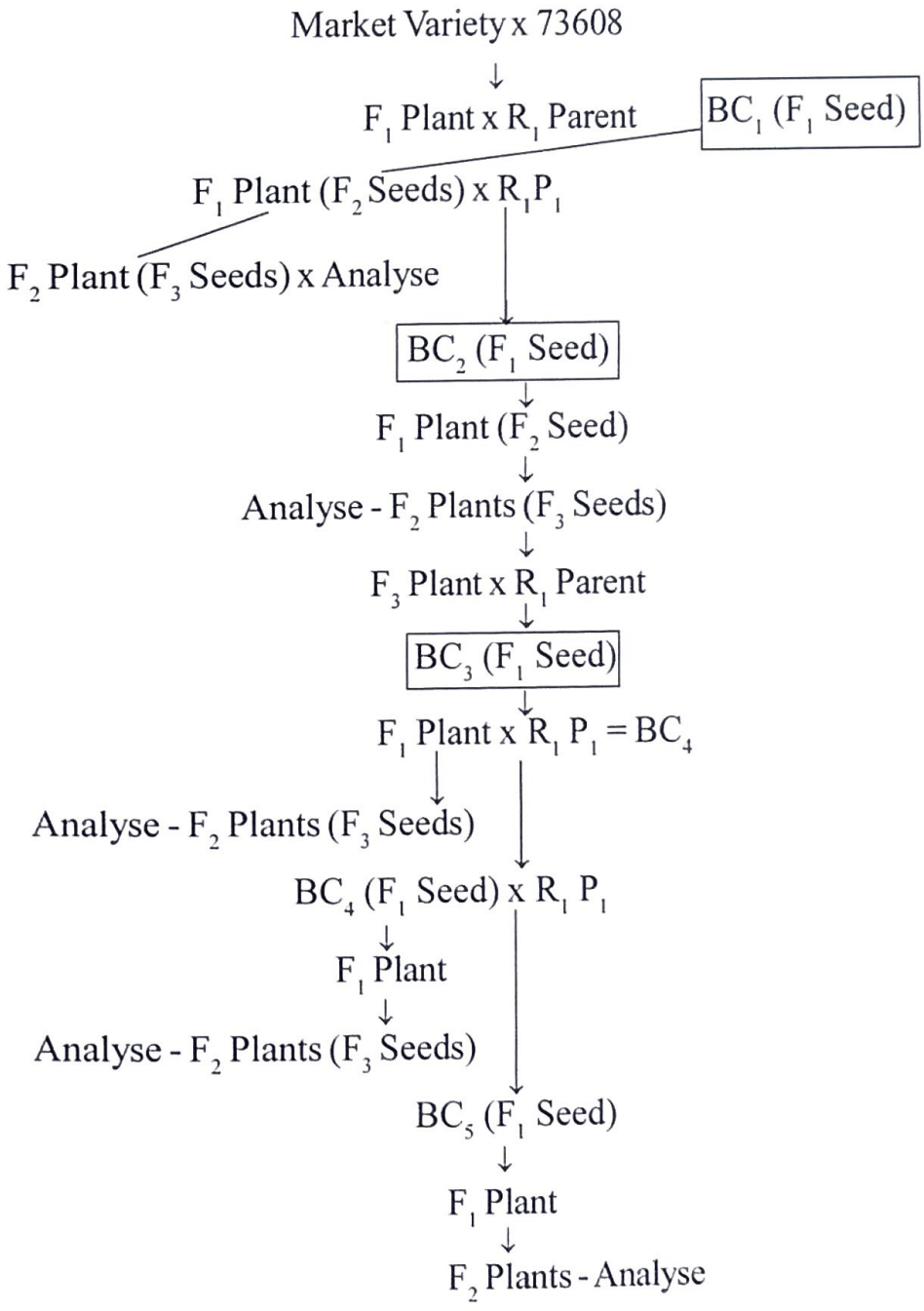

Market Variety x 73608
F1 Plant x R1 Parent
BC1 (F1 Seed)
F1 Plant (F2 Seeds) x R1P1
F2 Plant (F3 Seeds) x Analyse
BC2 (F1 Seed)
F1 Plant (F2 Seed)
Analyse - F2 Plants (F3 Seeds)
F3 Plant x R1 Parent
BC3 (F1 Seed)
F1 Plant x R1 P1 = BC4
Analyse - F2 Plants (F3 Seeds)
BC4 (F1 Seed) x R1 P1
F1 Plant
Analyse - F2 Plants (F3 Seeds)
BC5 (F1 Seed)
F1 Plant
F2 Plants - Analyse

Fig 1: Breeding Schedule

Rice

Rice has a balanced amino acid profile for its protein and this balance is not altered by increasing the protein percentage even by one or two per cent. This is because rice has low prolamine and high glutelin fraction compared to other cereal proteins (Nelson, 1979). The main task of quality improvement in rice is to increase the quantity. This is much easier than improving protein quality. Improvement in the protein content of rice through breeding would be valuable in view of the low nutritional status of people receiving 10 per cent or more of their calories from rice. Genetic variability for protein content has been generated through recombination and mutation breeding.

The extensive screening of germplasm (Coffman and Juliano 1979; Khush and Juliano, 1984) and induced mutants (Gangadharan *et al.* 1982; Ratho *et al.*, 1984) has led to the selection of high protein genotypes which have been utilised in breeding programmes for protein enhancement in rice. The increase in protein content of rice results in an increase in the number of protein bodies in the endosperm. The distribution of these protein bodies is more uniform and deeper in high protein selections than that in low protein varieties (Govindaswamy and Ghosh, 1974). The deeper distribution of protein bodies minimises the loss of protein during milling and polishing.

It has been reported by Ghosh and Sampath (1977) that a high protein (11.5 per cent) line, CRPH.8 has been selected from a cross between two high protein varieties (Pirurutong × Gaisen Mochi). This line with stable protein content was found to be poor yielder. For further improvement in yield, a backcross programme was followed with semi-dwarf variety Padma as the recurrent parent. CRHP.l, a selection from this backcross outyielded Padma in grain yield and showed 2 per cent more protein but gave less yield than IR 8. Selections have also been made from six crosses showing variation in protein content from 4.4-13.6 per cent (Gangadharan *et al.*, 1982).

Maize

Initially both *opaque-2* and *floury-2* genes were used for improvement of protein quality of maize. However, the use of *floury-2* was dropped and *opaque-2* was extensively used in the development of quality protein maize. A wide range of normal maize genotypes available in many laboratories have been converted to *opaque-2* using the traditional backcross approach. As a result, many *opaque-2* versions possessing original soft and chalky kernel characteristics are now available. Work on genetic modifiers was also pursued vigorously to investigate their role in altering kernel phenotype and biochemical characteristics of modified kernels (Annapurna and Reddy, 1971; Bjarnason *et al.*, 1976; Lodha *et al.*, 1976; Paez *et al.*, 1969). Through conscious research

efforts, using the modifying genes (genes that do not have any effect of their own, but they increase or decrease the expression of other oligo-genes), it has been possible to convert the phenotype of soft opaques vitreous horny-type endosperm (Vasal *et al.*, 1984).

Analytical methods

Availability of fast and reliable methods for screening protein content and quality are indispensable for breeding effort aimed at improving quality. Developments in the last decade have provided fast and reliable methods for protein estimation to the plant breeders. Grain protein analysers, which are based on infra-red reflectance measurement of the ground sample, offer great promise because of the speed of analysis. With these instruments 200-300 samples per day can be analysed simultaneously for protein content and moisture in the grain and screening is permitted not only of germplasm material but also of single plants in M_2 and M_3 generations of mutagenised population. However, in spite of lack of speed, the classical Kjeldahl method of nitrogen estimation continues to be the method of choice with most plant breeders because of simplicity, reliability and low cost of the equipment. More recently, equipments have been developed which have automated the entire estimation of nitrogen both at micro and macro levels. The Kjeltech system offers this advantage. With this unit, 60-80 analyses per day can be performed.

The other available rapid screening method is the Dye Binding Capacity Technique (DBC) which is based on the principle of binding of Acilan Orange G dye to flour protein. The amount of protein is proportional to the amount of dye bound. The method is useful for rapid screening of barley, wheat, maize and rice samples. For other grains the presence of phenols interfere with the correct assay.

Among the methods available, the fastest has been the screening by DBC technique. Also, since the dye binds to basic amino acids, DBC values provide relative idea about basic amino acids provided the data are expressed on relative nitrogen basis. This has often helped in the identification of mutants with elevated lysine content. The procedure requires, however, that N is estimated by Kjeldahl method. In actual practice one selects high lysine genotypes by plotting N vs DBC value and picks up positive deviates from the regression line. The deviates are then checked for content of lysine by amino acid analyser.

Chemical methods for determining contents of individual amino acid like lysine, methionine and tryptophan based on colorimetric estimation, have also been developed. Another procedure for estimation of essential amino acids, is the microbiological method. Antinutritional factors like trypsin inhibitors can be estimated by chemical method (Kakade *et al.*, 1969).

The chemical tests provide values for content of protein and individual amino acid but do not give any idea of their utilisation because the latter is affected by several other constituents in the grain. Therefore, at the advanced stages of breeding all materials should be analysed for nutritional quality by rat feeding trials. It has been suggested that estimations like true digestibility (TD), biological value (BV) and feed efficiency ratio (FER) should be made (Eggum, 1973). Even though, sizeable material is required for undertaking these tests, they provide sound information about the ultimate nutritional quality of the product.

Criteria for selection

Setting out goals and determining priorities is crucial to the success of a breeding programme. In breeding for quality, yield can never be sacrificed. Considering protein specifically, both the quantity and quality are of importance and the relative emphasis to be given to the two aspects will depend upon the target material. For example, in all cereals, except rice, protein quality improvement deserves priority because protein percentage found even in the present genotypes of cereals would adequately meet the protein requirement provided the proteins have a balanced amino acid composition. Feeding trials have shown 1.6 times higher weight gain and 40 per cent higher liver weights of rats fed on opaque-2 maize diet as compared to those fed on normal maize diet (Chinchalkar and Mehta, 1978). It is, therefore, essential to upgrade protein quality of cereal grains especially maize, barley and sorghum and high lysine genotypes are already available. In other grains like wheat where high lysine genotypes are not available, the first priority is to upgrade protein content. In rice also protein content need upgrading since in the polished rice protein content is very low. A search will have to be mounted for genotypes of rice in which protein distribution is more uniform across the grain so that milling and polishing do not deplete protein. Variability in distribution of protein across the grain has already been observed (Kaul *et al.*, 1973) and by suitable conventional breeding methods, it should be possible to incorporate this trait into commercial varieties. From the nutritional point of view 10 per cent protein with balanced EAA is better than even 15 per cent protein with imbalanced EAA. This point is of relevance because excess protein with imbalanced amino acid pattern puts a strain on energy, the unutilised amino acids can not be stored in the body and have to be excreted through a energy consuming process.

Pulses are rich in protein and there is no logic in attempting to increase it further unless the photosynthetic capacity can be substantially improved. At the current levels of photosynthetic capacity increased protein content will be only at the cost of yield loss. Higher levels of methionine and cysteine in the pulse protein, however, need to be looked for. Studies carried out so far have indicated that

among the two major storage proteins, namely legumin and vicilin, vicilin has better methionine content. Efforts should be made to improve its content through the use of suitable selection criteria. This has not been achieved so far because of the pre-occupation of plant breeders in increasing yield of pulses.

In terms of bioenergetics, much higher energy is required for protein synthesis compared to starch synthesis. Therefore, unless photosynthetic efficiency/ capacity also is increased, any net increase in protein would adversely affect starch accumulation and thereby yield. Relatively more protein is synthesised during early stages of grain development while more of starch is deposited during later stages of grain development. Although both protein and starch accumulations are subject to environmental influences, it is the starch which is influenced to a greater extent than protein. The variations in protein percentages of the same genotype during different seasons and at different locations arise mainly on account of changes in starch deposition rather than in protein. This, therefore, calls for separate criteria for selection of genotypes. Protein content per grain, although ideal, suffers from the drawback that in genotypes where grain number per spike is reduced, protein as well starch per grain tends to increase but offers no advantage. The best approach, therefore, is to take into account protein per grain and identify genotypes, that have inherent higher protein synthesising capability. This can be done by comparing protein/plant. Increase in protein/grain should not be at the cost of either grain number/spike or tillering. Although protein percentage in the final product is important from the nutritional point of view yet for breeding and selection, expression of protein on percentage basis could be deceptive. The failure of crosses of Atlas 66 with Indian wheats to yield lines with increased protein content is a reflection of this cause and is the consequence of the Indian varieties already having higher protein synthesising capacity than Atlas 66.

Another presently difficult looking approach is to examine the kinetics of protein accumulation in different genotypes which differ in protein percentage, grain weight and grain yield. Such studies in wheat have shown differential pattern of protein accumulation in bold seeded and low-grain weight varieties. The consistency of the pattern over years indicates that the pattern of accumulation is under genetic control. Therefore, it is possible to improve protein content by combining increased protein synthesis rate during early development of one strain with increased protein accumulation towards later stages of another. This certainly would require that biochemists and plant breeders work together—the ultimate rewards will amply justify it.

Constraints and difficulties in breeding for protein improvement

Even after two decades of identification of opaque-2 gene in maize and other high lysine genes in barley and sorghum, commercial varieties with improved protein quality have not been released in any cereal grain. An analysis of reasons for this situation is relevant. Most workers have observed negative correlation between improved protein quality and grain yield. Protein content/grain in most high lysine genotypes, in fact, has not increased but has either remained unchanged in some and actually decreased in most cases. Biochemical investigations have established clearly that yield decrease is largely contributed by a major decrease in starch (Mehta *et al.*, 1979; Batra *et al.*, 1982). From bioenergetic considerations there should be no yield penalty. On the contrary decrease in protein content should result in increased yield. However, this has not been the case. Therefore, unless this dilemma is resolved, conventional plant breeding will not succeed in protein quality improvement. The major objective of delinking protein quality improvement from starch synthesis would require an understanding of regulation of starch and protein biosynthesis. There are only a few groups working in this area and their work does not suggest single metabolic control. In high lysine Notch-2 for example, yield decrease is caused by the presence of a cavity in the grain (Fig. 2 a-b). In addition, modification of starch also leads to decreased starch biosynthesis (Fig. 2. c-d). The causation of cavity in the grain is not known and unless the cavity is removed, yield increase in Notch-2 is not possible. In maize and sorghum, air gaps and decreased size of starch granules may be the cause of decreased starch although evidence indicates that decreased level of starch synthesising enzymes, especially starch synthetase and phospho-keto isomerase, play an important role (Joshi *et al.*, 1980; Batra and Mehta, 1981a, b). It is, therefore, essential to generate information about the regulatory mechanisms controlling starch biosynthesis because without this basic information combining yield and improved protein quality would be difficult, if not impossible.

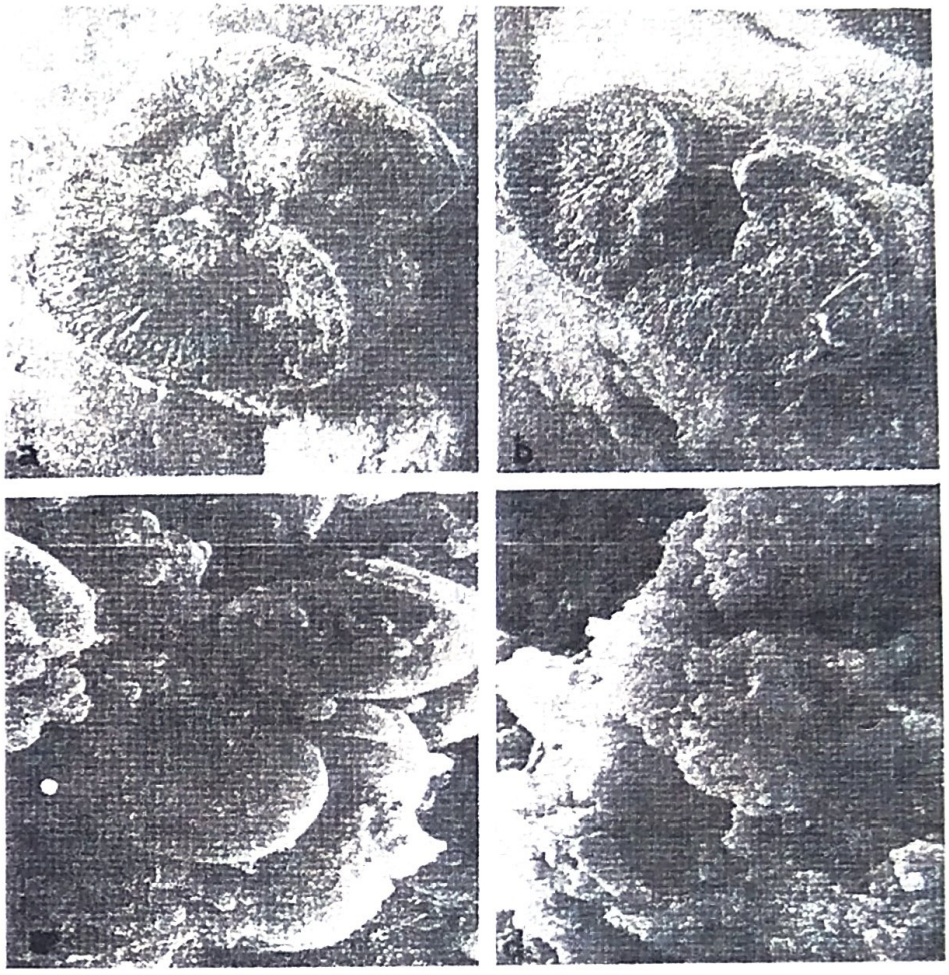


Fig 2, (a-d) Scanning electronmicrograph of T.S. of NP 113 (a) and Notch-2 (b) grains at 24 days after anthesis. The cavity in the centre of mutant Notch-2 grain is clearly visible.

Scanning electronmicrograph from the central endosperm region of NP 113 (c) and Notch-2 (d) from mature grains. Characteristic large and small type of starch grains in NP 113 are seen while in Notch-2 starch is seen as amorphous mass with irregular geometry.

Future strategies

While the conventional plant breeding for protein quality improvement would continue to play its role, recent developments in recombinant DNA technology offer greater opportunities for rapid progress in protein quality improvement in major cereals and pulses. We are now beginning to understand gene organisation for storage proteins in cereals and pulses. Major storage protein genes have been identified and sequenced. This, therefore, affords opportunity of *in vitro* mutagenesis for increased levels of limiting essential amino acids and reinsertion of mutated gene for expression in the host. The development of new plant gene cloning vectors allows re-insertion and expression of genes via protoplast. It

has been shown that a zein gene when incorporated with T-DNA into sunflower genome is transcribed. Similarly bean storage protein gene for Phaseolin is transcribed from its own promoter in transformed sunflower cell. However, while working with genetically restructuring plant storage protein genes, it must be kept in view that the seed storage proteins manufactured by plant are for supporting young embryo during germination and any change in protein constitution should be compatible with this requirement.

Another approach is to engineer microbes to synthesise storage protein for human food. From what has been achieved in synthesis of insulin, interferon and growth promoting substances, it appears possible that in not too distant future it may be possible to exploit genes for nutritionally improved storage proteins.

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8

Fruit Trees

P.K. Majumder

Most varieties of fruit plants have originated as chance seedlings or through somatic mutation and have been maintained and multiplied through vegetative propagation by fruit growers. The improvement of fruit plants through breeding has not yet attracted the serious attention of the research workers. Unlike field crops, fruit plant breeding is time consuming and laborious. Studies on the mode of inheritance of important economic characters of fruit plants are difficult because of the heterozygous nature of the plants. Moreover, varying degrees of sexual incompatibility have complicated the situation. Due to a long juvenile phase, it takes a long time to identify a useful hybrid. This factor has become a serious handicap because most research workers leave or shift from the programme before tangible results are obtained.

During the last two decades, considerable useful information has been generated on breeding methodology for the improvement of some of the important fruit crops. In this chapter fruits like mango, banana and papaya have been included.

Mango (*Mangifera indica* L.)

The genus *Mangifera* originated in South East Asia and is reported to contain 41 species (Mukherjee, 1949; Majumder and Sharma (1985). All the species are now restricted to the forest area of Indo-Malaysian region. Besides wild spp., more than 1,000 cultivars are known to exist in India and elsewhere. The enormous mango germplasm is on the verge of extinction due to selective cultivation of only a few commercial types.

Incompatibility system

Mango is a highly cross-pollinated crop and is usually pollinated through insects. Although some workers (Dijkman and Suole, 1951) had suspected the existence of self-sterility in mango, it was not confirmed until 1962 when Singh *et al.*

(1962) reported its occurrence for the first time in the cv. *dashehari*. Embryological studies (Mukherjee *et al.*, 1968) revealed that in the cvs. *dashehari* and *chawsa,* there was fertilisation after selfing as evidenced by endosperm growth, but after 15 days of pollination the endosperm degenerates completely and the fruitlets remain stunted. In the crossed flowers, on the other hand, the ovary is much enlarged (Table 1).

Table 1: Size of ovule on selfing and crossing (Mukherjee *et al.* 1968)

Cultivars	Size of ovule in mm	
	10 days after pollination	15 days after pollination
Dashehari (self)	1.00	1.02
Dashehari (cross)	1.05	3.75
Chawsa (self)	1.11	1.14
Chawsa (*cross*)	1.11	3.39

The embryological work also suggests that the pollen tubes grow down the style and effect fertilisation but the development of the zygote is blocked in a way similar to that found in cocoa (Dodds 1958). This type of incompatibility has also been observed in *Gasteria verrucosa* (Sears 1937) and in black currents (Ledeboer and Rietsema 1937). Mukherjee *et al.* (1968) Indicated that the self-incompatibility system in mango may be of sporophytic type. Sharma and Singh (1970) confirmed the findings of sporophytic nature of incompatibility in more elaborate studies on the cvs. *dashehari, chawsa, Bombay green* and *langra.* Pandey *et al.* (1972) showed that the level of auxin-like substances was higher in cross-pollinated fruitlets as compared to that of self-pollinated ones. The level of RNA and DNA was also found to be higher in the cross-pollinated fruitlets.

Breeding methodology

Gytological studies indicate that *Mangifera indica, M. sylvatica, M. caloneura, M. zeylanica, M. caesia, M. foetida* and *M. odorata* all possess 2n = 40 (n = 20) chromosomes (Mukherjee, 1950; Roy and Visweswariya 1951). The inter-specific crosses between *M. zyelanica* and *M. odorata* (Mukherjee, 1963) were found to be fertile which is a happy situation.

Techniques of hybridisation

During 1911-1960, large sized muslin bags having two to three iron rings were utilised in the hybridisation programme (Sen *et al.*, 1946). These were cumbersome in handling since each bag enclosing a panicle had to be opened

and closed everyday during the flowering period for making pollinations. Consequently, only a few panicles could be handled during the short flowering period. Also, the number of flowers pollinated on a single panicle ran into hundreds because the same panicles were utilised on a number of days. Since normally only one fruit is obtained per panicle, most of the effort was wasted.

Mukherjee *et al.* (1961) realised these difficulties and an improvement was introduced to increase the efficiency of mango hybridisation work. Emphasis was laid on utilising a large number of panicles and pollinating a few flowers per panicle. The conventional muslin bags were replaced by light (100 gauge thickness) small sized polythene bags which were finely perforated before use. With this simple technique mango hybridisation work was revolutionised.

The discovery of self-incompatibility in some of the most popular cultivars of mango, led to the standardisation of the caging technique in which pollination was effected through freshly bred houseflies thus doing away with hand pollination (Singh *et al.*, 1977). The technique of Mukherjee *et al.* (1961) was further improved by doing away with rebagging after pollination (Singh *et al.*, 1980).

Hybridisation and production of new cultivars

Attempts to develop improved cultivars of mango through breeding have been underway since the beginning of the present century. An account of the work done in different locations using old and new techniques, is presented in Table 2.

Table 2: Hybridisation work undertaken in mango at different locations

S. No.	Reported by	Location and period	Total flowers crossed	Fruits obtained	
				Total	Per year
Old Technique					
1.	Burns and Prayag (1921)	Poona (1911-14)	494	—	—
2.	Sen *et al.* (1946)	Sabour (1941-44)	9,737	77	19.25
3.	Rao *et al.* (1958)	Kodur –(1941-58)	53,000	239	13.27
4.	Jawanda and Singh (1963)	Qadian (1941-58)	7,775	31	3.87
5.	Singh (1963)	Saharanpur (1951-62)	25,129	59	4.91
New Technique					
6.	Mukherjee *et al.* (1961)	Krishnagar (1959-61)	26,911	362	120.66
7.	Majumder and Sharma (1985)	IARI (1961-81)	82,000	1,252	59.61

The work at Sabour yielded two promising hybrids namely, "Mahmud Bahar" and "Prova Sankar". However, nothing was heard about these subsequently

(Sen *et al.*, 1946). The situation is similar about the hybrids "Neeluddin", "Swarna-Jehangir", "Neelgoa" and "Neelashan" (Rao and Rangacharlu, 1958) evolved at Kodur. The hybridisation work carried out at the IARI led to the evolution of two improved regular bearing hybrids namely "Mallika" and "Amrapali". Amrapali is of dwarf, precocious and high yielding. It has been utilised for a high density plantation which has given exceptionally high yield per unit area (Majumder *et al.*, 1982). Besides, Amrapali possesses very high vitamin A content (β carotenoid pigment) because of which it has a deep orange flesh colour. The deep flesh colour is important in fruit preservation industry, and addition of artificial colour is not required. Salvi (1983), from Maharashtra, also reported the evolution of 'Ratna' from Alphonso Neelum cross.

Pre-selection method

In a long lived perennial with a long pre-bearing age, it is advantageous to identify characters which can facilitate selection of the desired types among the hybrid population at an early stage. This can save precious time, land and labour. Indicative criteria which could give of precise indication of the fruit quality, bearing habit and growth habit have now been identified. Leaf flavour has distinct correlation with the fruit flavour (Majumder *et al.*, 1972). The emergence of new growth flushes, simultaneously with fruiting or immediately after harvest is indicative of regularity of bearing (Sharma *et al.*, 1972). Likewise, a lower stomatal density per unit area of abaxial leaf surface is indicative of dwarfness (Beakbane and Majumder, 1975).

Selection of parents

Of the three regular bearing parents *neelum, totapari red small* and *bangalora,* used in hybridisation programme by Sharma and Majumder (1985), the cv. *neelum* was found to be a better combiner. In the crosses involving *totapari red small* and *bangalora,* the regular bearing hybrids invariably had inferior fruit. This indicated negative linkage between regularity of bearing and fruit quality.

Use of induced mutations

A plant breeder's approach towards finding the solution for major problems in a crop like mango is largely centred around creation of wide genetic variability and its utilisation, besides screening of existing germplasm. This wide genetic base can be created in two ways: (i) through conventional hybridisation method, and (ii) by induction of mutations. Mutation breeding which holds great promise has not attracted the attention of mango breeders. Sharma *et al.* (1983) made serious efforts of harnessing the advantages of induced mutagenesis by using physical (γ-rays) and chemical mutagens (EMS and NMU). The treated scions

were grafted *in situ* for the recovery of mutants. It has been observed that dosages of γ-irradiation beyond five kR are lethal to mango. The LD_{50} for *neelum, dashehari, amrapali* and *mallika* was 3.9, 2.9, 3.2 and 2.4 kR, respectively. The effective dosages of ethyl methane sulphonate (EMS) and N-nitrosomethyl urea (NMU) were found to be 1.50 per cent and 0.05 per cent respectively (Sharma *et al.*, 1983).

The spectrum of changes was more or less the same with both types of mutagens. The abnormalities noticed in vM_1 generation were smaller and larger leaves, lanceolate leaves, ovate leaves, and leaves with deeply cut margins, twisting mid-ribs and disturbed phyllotaxy (Sharma *et al.*, 1983).

Repeated heading back of the plants obtained from mutagen treated scions resulted in stabilisation of characters like small leaves and dwarf habit, and leaves completely different from the cv. *dashehari.* The latter mutant had exceptionally high TSS (25°Brix). The dwarf mutant can fit in high density planting (Sharma and Majumder, 1985a).

Pattern of genetic control

Information on this aspect was not available until recently. An insight has now been obtained into the inheritance of characters like tree habit, juvenility, regularity of bearing, fruit quality, fruit size, fruiting in bunches, fruit beak, colour of new leaves and panicles, fruit colour and resistance to diseases (Majumder *et al.*, 1972; Sharma *et al.*, 1972; Singh *et al.*, 1977; Sharma and Majumder, 1985).

Fruit size is an important character which is governed by polygenes. Observations on fruit size in five parental combinations involving the cvs. *neelum, totapari red small, bangalora* as parents indicated that the fruit size of the hybrids, in general, was inferior (Table 3). However some hybrids in all combinations showed increased size over the better parent (Sharma and Majumder, 1985).

Table 3: Transmission of fruit size in mango crosses (Sharma and Majumder, 1985)

Parental combination	Total Hybrids studied	No. of hybrids with fruit size of the type			
		Parental		Larger than better parent	Smaller than inferio parent (-1)
		0 +	0	+	
D × T	68	21	12	7	28
D × N	129	33	17	5	74
N × D	29	3	3	3	20
N × C	21	3	7	2	9
D × B	55	21	18	21	14

D = Dashehari; T = Totapari Red Small; N = Neelum; C = Chawsa; B = Bangalora.

The hybrids from crosses involving red skin coloured (cv. *sensation, totapari red small* and green skin coloured (cv. *dashehari* and *amrapali)* fruits revealed that red skin colour is dominant (Sharma and Majumder, 1985).

The presence of beak and sinus are considered to be undesirable characters. Unfortunately, these two characters are governed by dominant genes and the cultivar *totapari red small* and *neelum* possess the characters. All the hybrids involving the above cultivars invariably inherited these two characters suggesting that these characters are homozygous in the parents (Majumder *et al.*, 1972).

Amongst the regular bearing cultivars utilised in hybridisation, *neelum* is susceptible to bacterial canker. In whichever combination this cultivar was used as female parent, the incidence of this disease was invariably noticed (Table 4) (Sharma and Majumder, 1985). This is indicative of cytoplasmic control of this disease. This fact needs to be kept in mind by mango breeders.

Table 4. Transmission of 'bacterial canker' in mango crosses (Sharma and Majumder, 1985)

Parental combination	Number of hybrids and the extent of disease incidence				
	Severe	Medium	Mild	Free	Total
Dashehari × *Neelum*	-	-	14	180	194
Neelum × *Dashehari*	51	20	5	-	76
Neelum × *Langra*	28	-	-	-	28
Neelum × *Chawsa*	18	10	4	1	33
Neelum × *Safdar Pasand*	15	4	3	1	23
Neelum × *Himsagar*	20	4	2	-	26

Banana (*Musa* spp.)

The family *Musaceae* has two genera namely, *Ensete* and *Musa*. The genus *Ensete* comprises monocarpic herbs, none of which bears edible fruits. The most important species is *E. ventricosum* which yields useful fibre and starchy food. The genus *Musa* is divided into five sections namely: (1) *Eumusa,* (2) *Rhodochlamys,* (3) *Callimusa,* (4) *Australimusa,* and (5) *Incerte sedis*. The important characters of the section are given below:

1) *Eumusa* (x = 11; 2n = 22 or 33): This is the largest section with many species. The characteristics of this section are pendent or semi-pendent inflorescences, dull coloured bracts and many flowers in two rows in each bract. The edible cultivated parthenocarpic bananas belong to this section and are derived from interspecific cross between *M. acuminata* and *M. balbisiana*. The other important spp. are *M. itinerans, M. flaviflora, M. laterita, M. basjoo* and *M. nagensium*.

2) *Rhodochlamys* (x = 11; 2n = 22): Inflorescence erect flowers few to a bract usually in a single row; parthenocarpy absent. *M. ornata* and *M. velutina* are sometimes grown as ornamental plants. The other spp. are *M. laterita, M. ornata, M. sanguinea* and *M.rubra.*

3) *Callimusa* (x = 10; 2n = 20): Plants are small in stature, usually with erect inflorescence with purplish bracts, parthenocarpy absent. *M. coccinea* is grown as ornamental plants.

4) *Australimusa* (x = 10; 2n = 20): Inflorescence pendent or semi-pendent. The most important species of this section is *M. textilis* also called abaca or Manila hemp, which supplies important fibres of commerce. *M. maclayi* (Fe'i banana) is still eaten in Polynesian islands after cooking. The fruits are parthenocarpic and predominantly female sterile; fruiting bunch is erect and contains red sap; fruit skin is orange in colour when ripe and flesh is yellow. The other spp. are *M. peckelli, M. lododensis* and *M. angustigemma.*

5) *Incertae sedis:* This section has a few species of doubtful affinity. Among these is *M. ingens* (x = 7; 2n = 14), which was discovered in New Guinea and grows to a height of over 10 m and *M. baccari* (x = 9; 2n = 18).

Diversity of edible bananas

The banana is believed to have originated in hot tropical regions of South-east Asia (Simmonds, 1962). *Eumusa* series contains a great majority of banana cultivars and has bispecific origin from *M. acuminata* and *M. balbisiana.* The diversity of *Eumusa* cultivars are shown in Table 5.

Table 5. Diversity of edible bananas

Genome	Centre of origin	Present diversity
AA	Malaysia	Malaysia and coastal east Africa
AAA	Malaysia	Diversified by mutation; secondary variability in East Africa
AB	India	Negligible Elsewhere
AAB	India and Eastern Malaysia	Secondary diversification in West Africa
ABB	India and Eastern Malaysia	Negligible elsewhere
ABBB	Indochina	Nil

Germplasm and its evaluation

Several Latin names have been used till recently in the botanical nomenclature of the banana, three of the earliest employed being (1) *M. paradisiaca,* (2) *M. sapientum,* and (3) *M. cavendishii.* These are not species but refer to closely allied triploid interspecific hybrids. Kurz in as early as 1865 recognised the

essential fact of bispecific origin of the cultivated banana which was later confirmed by Cheeman (1948); Dodds and Simmonds (1948). The cultivars are now recognised on the basis of their genomic constitution as, for example, *Musa* AAA or AAB group.

The world germplasm of banana is enormous. Some of the important Indian germplasms of banana are as follows:

AA group: (1) *Chingan*; (2) *Matti*; (3) *Anaikomban*; (4) *Kadali*; (5) *Nomarai*; (6) *Suryakadali*.

AAA group: (1) *Amritsagar*; (2) *Basrai*; (3) *Giant Cavendish*; (4) *Robusta* (*Harichal*); (5) *Chakrakeli*; (6) *Agniswar* (*Lalkela*).

AB group: (1) *Ney poovan (Safetvelchi);* (2) *Kunan.* AAB group: (1) *Poovan (Champa);* (2) *Rasthali (Martaman);* (3) *Nendrapadaththi;* (4) *Sirumalai (Virupakshi);* (5) *Nendran;* (6) *Rajapuri.*

ABB group: (1) *Monthan (Kanchkela);* (2) *Nalla bontha;* (3) *Nalla bontha bathees;* (4) *ThenKunan;* (5) *Thattila Kunan;* (6) *Peyon.*

Analysis of germplasm

The cultivars of Musa AAB, plantain subgroup, are of particular interest in West African countries where plantains are the staple food. Being a secondary centre of origin, enormous germplasm exist in West Africa. According to Majumder (1980), the plantain group can be subdivided into four main series:

1) Horn: Male bud absent (Fig. 1).
2) False-Horn: Male bud degenerate at ripening, male axis with few neutral flowers (Fig. 2).
3) French-Horn: Male bud degenerates at ripening, male axis with many neutral flowers (Fig. 3).
4) French plantain: Male bud present at ripening, male axis with all the neutral flowers usually with persistent bracts (Fig. 4).

The West African germplasm was analysed (Majumder, 1980) using Hutchinson's Polygraph technique in Xanthia (Love and Nadeau, 1961; Majumder and Mukherjee, 1972) and 14 distinct phenotypes were identified from a large number of cultivars (Fig. 5). Twelve distinct heritable characters were chosen for drawing the polygraph. These are: (1) Height; (2) Pseudostem colour; (3) Petiole colour; (4) Petiole margin colour; (5) Lower midrib colour; (6) Petiole margin opening; (7) Bunch position; (8) Male bud character (9) Fruit position; (10) Fruit length; (11) Number of hands; and (12) Fruit apex were chosen for drawing polygraph (Fig. 6).

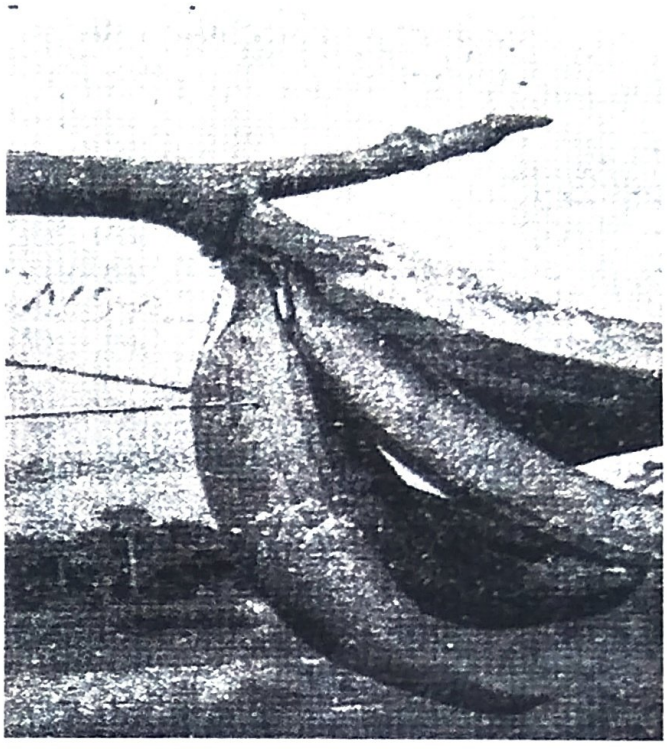

Fig. 1: Horn plantain-male bud absent.

Fig. 2: False-Horn plantain-male bud degenerates at ripening, male axis with few neutral flowers.

Fig. 3. French-Horn plantain-male bud degenerates at ripening, male axis with many neutral flowers.

Fig. 4. French plantain-male bud present at ripening, male-axis with all the neutral flowers along with bracts.

Reaction of genomes to diseases

Panama wilt (*Fusarium oxysporum* Cubense), which has wiped out the cultivation of cv. *Gros Michel* in Jamaica, has been found to react differently with different genomes. The resistant genotypes are of great commercial significance. Clonal reaction to panama disease is shown in Table 6 (Anonymous 1954; Simmonds, 1966; Khader *et al.* 1985).

Another important fungal disease is leafspot or Sigatoka (*Cercospora musae*—asexual stage and *Mycosphaerella mucicola*—sexual stage). The intensity of the disease is sometimes so severe that the crop is nearly lost. It is of great economic interest to select cultivars resistant to this disease. All triploid AAA bananas of commerce are highly susceptible to leafspot disease, while all ABB clones are resistant. The contribution of 'B' genome towards resistance appears significant. Table 7 gives the pattern of genomic reaction (Anonymous, 1954; Simmonds, 1966; Majumder, 1980; Khader *et al.* (1985).

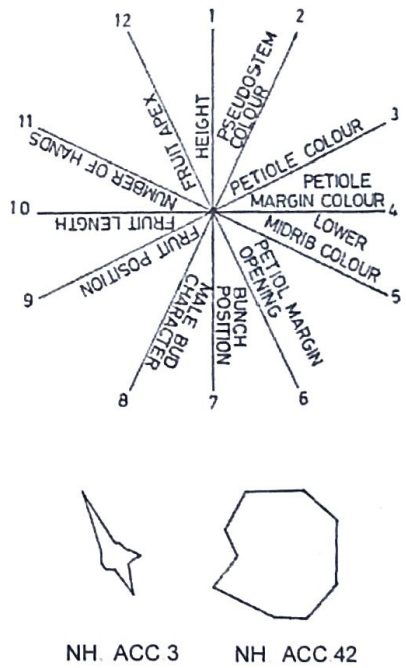

Fig. 5. Key of polygraph AAB.

Genomic reaction to frost

Banana is a tropical plant and cannot tolerate low temperature during growth. Several banana clones, comprising different genomes, were subjected to low temperature condition prevailing in Delhi and the genomic response to 19 frosty nights was determined (Venkatesam, 1966). The 'B' genome appeared to have greater contribution towards frost resistance.

Breeding behaviour of wild species

Several references have been made about the occurrence of natural inter-specific hybrids among the wild bananas. The crossability of *Musa* species has been reported by Simmonds (1962). The salient features are summarised in the following:

Musa acuminata crosses easily with *M. basjoo,* but many empty seeds occur and germination is poor; reciprocal crosses are inviable. *M. balbisiana* crosses easily with *M. laterita, M. ornata, M. sanguinea, M. velutina* and the hybrids are viable.

Species/cultivars	Genomic constitution	Reaction to frost
M. balbisiana	BB	Resistant
Kunan	AB	Resistant
Pocha bontha bathees	ABB	Resistant
Monthan	ABB	Resistant
Rasthali	AAB	Fairly resistant
Champa	AAB	Fairly resistant
Lalkela	AAA	Very susceptible
Basrai	AAA	Very susceptible

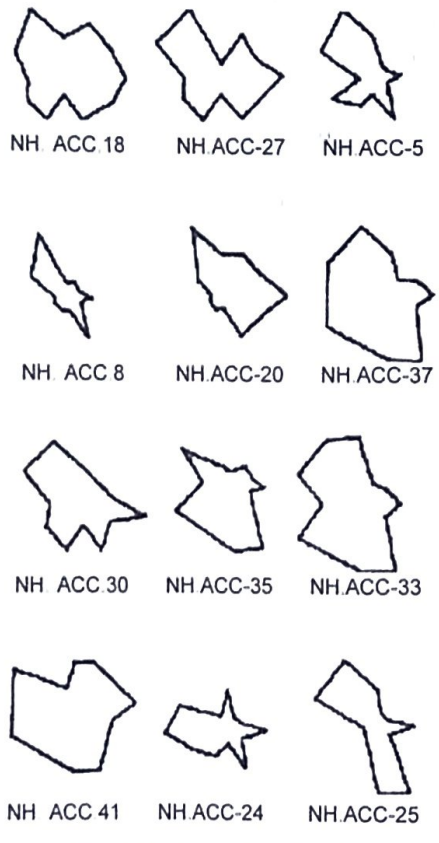

Fig. 6: Polygraphs of AAB.

Table 6. Clonal reaction to panama disease in banana

Group	Cultivars giving the reaction grade			
	1	2	3	4
AA	—	Bonde Michare	Paka	Pisang Lilin, Tongat, Sucrier, Sikuzani, Palembang
AAA	Gros Michel Lai Kel Amritsagar	Green Red	Rio	Basrai, Giant Cavendish, Robusta, Orotava, Marathuba
AAAA (Bredcultivar)	—	—	IC_2	Bodies Altafort
AB	—	—	—	Ney Poo van, Kunan
AAB	Silk, Sonkel, Rasthali, Malbhog	Padaththi Kanthali, Virupakshi	Pome, Nadan	Poovan, Nendran, Plantain sub-group, Pisang Rajah
ABB	—	Peyan	Pisang awak	Bluggoe, Monthan, Pocha bontha bathees
ABBB	—	—	—	Klue Teparod

1: Susceptible; 4: Resistant.

Table 7. Leafspot reaction on different genome

Group	Cultivars giving the reaction grade			
	1	2	3	4
AA	Sucrier, Bonde	—	Michare	Tongat, Pisang Lilin, Palembang, Paka
AAA	Cavendish Group, Gros Michel, Marathuba	Rio	Red banana, Green red	Orotava
AAAA	—	—	—	IC_2
AB	—	—	—	Ney Poovan, Kunan
AAB	—	Nendra Padaththi	Rasthali Pisang Rajah, Pome, Nadan, Silk	Poovan, Plantain subgroup
ABB	—	—	—	Bluggoe, Peyan, Pocha Bontha Bathees, Monthan, Nalla bontha, Then Kunan Thattila Kunan
ABBB	—	—	—	Klue Teparod

1: Susceptible; 4: Resistant

M. basjoo crosses easily with *M. laterita, M. ornata, M. sanguinea,* and *M. itinerans* whereas the reciprocal crosses are infertile. *M. itinerans* also crosses easily with *M. laterita, M. ornata, M. sanguinea, M. velutina* and the

reciprocal crosses are nonviable. Similarly *M. laterita* crosses easily with *M. ornata* and *M. velutina* whereas with *M. sanguinea* it is sterile.

Hybridisation and varietal improvement

Banana breeding was first started at the Imperial College of Agriculture, Trinidad in 1922. The main objective was to incorporate resistance to Panama disease and leafspot into Gros Michel. Gros Michel was first crossed, as female parent, with wild seeded strains of *M. acuminata* sub spp. *malacensis*. The hybrids IC_2 and S_{19} (both AAAA) developed from this cross were edible and resistant to both the diseases but the bunch form had defects of the male parent and were not acceptable to the trade (Cheesman, 1949).

The Cavendish group (AAA) never sets seed when pollinated with diploid and therefore has not been used in a breeding programme. It was necessary, therefore, to search a male parent which is resistant to both the diseases, but produces abundant pollen and is female sterile. The edible diploid cv. Pisang lilin from Malaysia was chosen as male parent. One tetraploid hybrid clone, with moderate quality, was selected which was later named "Boddles Altafort". This too did not satisfy the trade (Cheesman, 1949). Menendez and Shepherd (1975) used a different breeding approach. A male parent with good bunch character was selected from the crosses between *banskii* and *malacensis*. Using this male parent, crosses were made with 'High Gate' which is a dwarf mutant of Gros Michel. Several useful hybrids, resistant to both the diseases were evolved. These are still under testing.

In India, the improvement of banana through breeding did not receive serious attention. Work at the Central Banana Research Station, Aduthurai (Tamil Nadu) was started in the early fifties and was later continued at the Agricultural College and Research Institute, Coimbatore. Out of 43 hybrids assessed (Krishnamurthi and Rao, 1964) one hybrid No. 135

$(\underset{AAB}{\text{Laden}} \times \underset{BB}{M.\text{balbisiane}} \times \underset{AA}{\text{Kadali}})$ was later released as CO_1.

Somatic mutation

It is well recognised that somatic mutations have contributed enormous diversity in bananas in different parts of the world. Mutations are known, or suspected, to have affected 18 characters. Amongst these, stature and habit, plant colour and fruit characters are of horticultural significance (Simmonds, 1966). The Dwarf Cavendish, Giant Cavendish and Robusta bananas are mutants of less widespread clones. These three clones jointly contribute a major share to the world banana crop and trade. High Gate is the dwarf sport of the popular Gros

Michel. Numerous mutants of AAB and ABB occur in Asia and Africa (Majumder 1980; Khader *et al.*, 1985). Mention may be made of the following:

Original clone	Mutant clone
Nendran (AAB)	Eleri
Rasthali (AAB)	Ayiranka Rasthali
Virupakshi (AAB)	Krishna Vazhai (Black stem)
Maiamaole (AAB)	Eleele, Mahoe
Maiden plantain (AAB)	Tiger plantain
Benin variety (AAB)	Dichotomous mutant (Fig. 7)
Poovan (AAB)	Mott poovan
Then Kuman (ABB)	Thattila Kuman (no male bud)
Monthan (ABB)	Pocha Bontha Bathees
Koroboi Chuku (ABB)	Koroboi miraba

Fig. 7. Dichotomous mutant-peduncle divides into two immediately after emergence making two bunches.

Although natural mutations have played a great role in the evolution of new cultivars, reports of induced mutations are rare. Volez and Cedeno (1972) obtained two mutants from Gros Michel using gamma irradiation. One had drastic leaf aberrations and the other had intense pigmentation. These have no commercial significance. Menendez (1973) applied EMS to the seeds of *M. acuminata* and obtained short stature mutants. Guzman *et al.* (1976) advocated the treatment of callus for the mutation work.

Genetic studies

The inheritance of parthenocarpy is an important genetical investigation and was carried out in crosses between seeded diploids and the edible diploids. In one group, parthenocarpy appeared to be controlled by a single dominant gene (P), the expression of which, however was affected by modifiers. In another case, however, inheritance was more complex (Dodds, 1943; Dodds and Simmonds, 1948a). Crosses of seedless diploid (*M. acuminata*)with edible diploid indicate that parthenocarpy is due to several dominant genes. Simmonds (1953) proposed the following constitution:

Pisang lilin	P_1p_1	P_2p_2	P_3p_3
M. malacensis	p_1p_1	P_2p_2	P_3p_3
M. burmannica (Long Tavoy)	p_1p_1	p_2p_2	P_3p_3

Simmonds has also suggested that the persistence of bracts and flowers on the male axis of the bunch is probably due to complimentary genes, which can express only in the presence of parthenocarpy. Persistence of bracts and parthenocarpy are associated in the inheritance and are pleotropic (Simmonds 1953).

Bract persistence is common in wild *M. balbisiana.* It occurs occasionally among established edible bananas including hybrid clones, Nendra padaththi (AAB) and *acuminata* clones, Dwarf Cavandish (AAA). The case of Dwarf Cavendish is of special interest because the bract persistence is one of a syndrome of characters which differentiate this mutant from the parent stock with fully deciduous bracts. A sister mutant, the Giant Cavendish, is intermediate between the dwarf and the normal in respect of both stature and bract persistence. The dwarfing and bract persistence are pleotropic aspects of a single genetic change (Simmonds, 1953).

Papaya (*Carica papaya* L.)

The papaya is a native of tropical America but is now grown in almost all tropical and subtropical regions of the world. Genus *Carica* has as many as 40 species. Of these, *Carica papaya* L. is cultivated.

Genetics of sex

Papaya has three distinct sex forms: (1) staminate or male; (2) hermaphrodite; and (3) pistillate. Of these male and hermaphrodites are not stable. The sex-reversing male and hermaphrodite readily change sex as a result of seasonal changes, particularly in temperature. However, pure male and pistillate are stable (Singh *et al.*, 1961; 1963).

Hofmeyr (1938) and Storey and Jones (1941) suggested that a single gene with three alleles controls sex-expression. According to this hypothesis, males and hermaphrodites are in heterozygous state while the female is in homozygous condition. The scheme for sex determination in papaya using the Mendelian symbol was first proposed by Hofmeyr (1938) and it was later adopted by Storey and Jones (1941). The genetic constitution of male, hermaphrodite and pistillate plants are as follows:

M_1m—Male plant

M_2m—Hermaphrodite plant

mm—Pistillate plant

Subsequently, Storey (1953) reported that sex in papaya is determined not by single gene but rather by a complex of genes which lie closely linked in different segments occupying identical regions on sex chromosomes. The sex determining segments behave in heredity as if they were unit factors. He further reported that there are two independent sets of factors which may modify sex-expression in males and hermaphrodites under certain environmental conditions. One set is responsible for seasonal shifts from female fertility to sterility and *vice versa*. The other set causes stamens to become carpelloid, usually with fusion to the pistil. Horovitz (1954) has attributed sexual variability to the presence of alleles F and F^h which are indispensable for anther formation. Within a given genotype, temperature is the decisive factor for the type of flower produced at a given moment. The existence of specific male and female florigenic substances has been postulated by him.

Hofmeyr (1938) overlooked the presence of two types of male: (i) pure male, and (ii) sex-reversing male. Pure male was never found to change sex even after repeated heading back, whereas sex-reversing males change sex as a result of seasonal change in temperature (Singh *et al.*, 1961). The genetic make-up of sex-reversing male was first determined by Ram *et al.* (1983, 1985). They found that when sex-reversing male was selfed, it gave a progeny of sex-reversing male and pure male in the proportion of 3 :1. On the other hand when sex-reversing male was crossed with pure male, the segregation ratio of sex-reversing male and pure male was 1:1. This suggests that multiple allelism is operating. Ram *et al.* (1983, 1985) have proposed the following symbols for sex-reversing and pure male on the basis of experimental findings:

$$\left.\begin{matrix} M_1^{RR}m \\ M_1^{Rr}m \end{matrix}\right\} \text{sex-reversing male}$$

$$\left.M_1^{rr}m\right\} \text{Pure male}$$

Attempts so far made to discover a sex linked character in seed or juvenile vegetative characters of seedling which might be useful in early detection of sexes, have not been successful (Higgins and Holt, 1941; Hofmeyr, 1938; 1939 and Storey, 1953). Singh *et al.* (1961a) predicted the sex of nursery seedlings by colorometric test and the forecast was accurate up to 88 per cent in the case of female and 67 per cent in the case of male. A number of cytological studies have been conducted to determine whether papaya has a heteromorphic pair of chromosomes (Lindsay, 1930; Hofmeyr, 1938). Meurman (1925) could not identify sex chromosomes in papaya. However, Kumar *et al.* (1945) reported precocious separation of one pair of chromosomes at anaphase-1 of meiosis in male and hermaphrodites. They did not observe such separation at anaphase-1 in the females. Storey (1953) also observed precocious separation in PMC of one pair of chromosomes in high frequency.

Breeding methodology

Since papaya is dioecious in nature, to establish a variety by breeding procedures is not easy. Inbreeding and selection are the main procedures adopted by various workers (Hofmeyr, 1933; 1936; Agnew, 1951). Two lines of breeding are being followed for improvement.

i) *Dioecious*: It has been established that female plants of papaya are more productive. Hermaphrodite plants are more influenced by environment and are also less productive (Singh *et al.*, 1963). Due to out-crossing, most of the cultivars are highly variable. For bringing about uniformity by homozygosity, sib-mating between male and female plants of a cultivar is recommended. For this purpose, desirable male and female plants are selected from a progeny which share identity of vegetative characters such as stem colour, petiole colour, height at flowering and are sib-mated. Seedlings raised from S_1 inbreds are screened and desired male and female plants are selected for further sib-mating. The process is continued for three to four generations to achieve uniformity for a group of characters. In this method, males and females will occur in equal proportion in the progeny.

ii) *Gynodioecious line*: This method has been profitably utilised in Hawaii for the production of 'Solo' types of papaya. The main advantage of this method of breeding is that all the plants are productive. Suitable hermaphrodite plants which are insensitive to climatic change, are selected for inbreeding. Of the various types of flowers produced by a hermaphrodite plant, elongata and pentandria types are selected for selfing. Selfing of selected hermaphrodite plant is continued for at least three generations for attaining uniformity of characters. As in the case of dioecious line breeding method, controlled pollination is necessary for seed production in this method also.

The only commercial cultivar that is relatively stable in performance is 'Solo' from Hawaii. Several other gynodioecious cultivars such as 'Sunrise Solo', Waimanalo, Higgins and Wilder were also developed at Hawaii (Nakasone *et al.*, 1972; 1974).

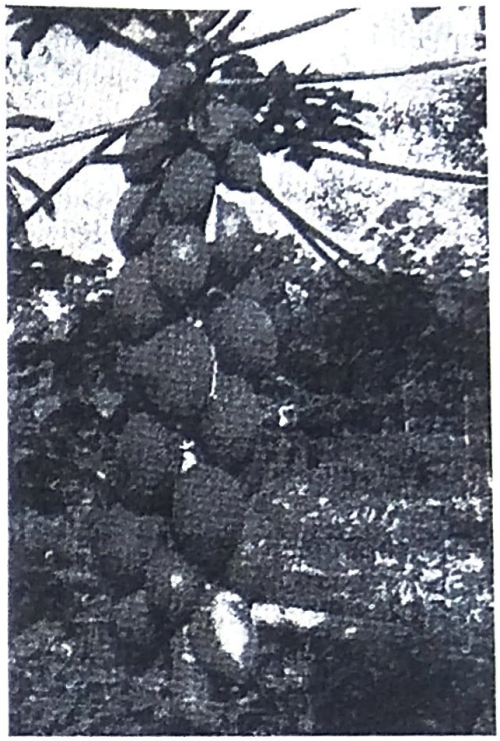

Fig. 8. C.P.L.-Dwarf and high yielding selection.

Ayappa and Nanjappa (1959) in Coorg selected Coorg Honey which is gynodioecious in nature. Two selections namely, CO1 and CO2 have been developed at Coimbatore by inbreeding of the cv. Ranchi for seven generations. Majumder (1980) selected dwarf, high yielding plant type (C.P.L.) with low fruiting height (Fig. 8) in Nigeria from Homestead papaya. The selection has dominant purple colour in all plant parts and serves as a marker gene.

At the IARI Regional Station, Pusa, separate lines of gynodioecious and dioecious from the cv. Ranchi have been developed. These have been released as varieties under the names Pusa Delicious, Pusa Majesty, Pusa Giant and Pusa Dwarf. Pusa Delicious and Pusa Majesty are gynodioecious while the other two are dioecious (Ram, 1981; 1982).

Heterosis breeding

Hamilton (1954) made elaborate selfing and crossing using dioecious and gynodioecious lines but did not observe either hybrid vigour or in breeding depression. Dai (I960) first reported heterosis in the cross between varieties 'Philippine' and 'Solo'. A high positive heterosis for fruit size and number of seeds was observed by Shah and Shanmugavelu (1975) in the cross CO1 × Coorg Honey at Coimbatore.

Iyer and Subramanium (1981) reported heterosis for all the vegetative characters, fruit yield and its components. Heterosis up to 111.41 per cent for yield was obtained in crosses Solo Yellow × Washington. Ram (1982a) has recorded marked heterotic response in crosses between Ramnagar Local and Washington and Pusa 1-15 × Halflong. No planned hybrid has reached a varietal status, however.

Mutation breeding

Ram and Majumder (1981) for the first time evolved dwarf mutant by treating seeds of papaya with 15 kR of gamma rays. Initially, three dwarf plants were

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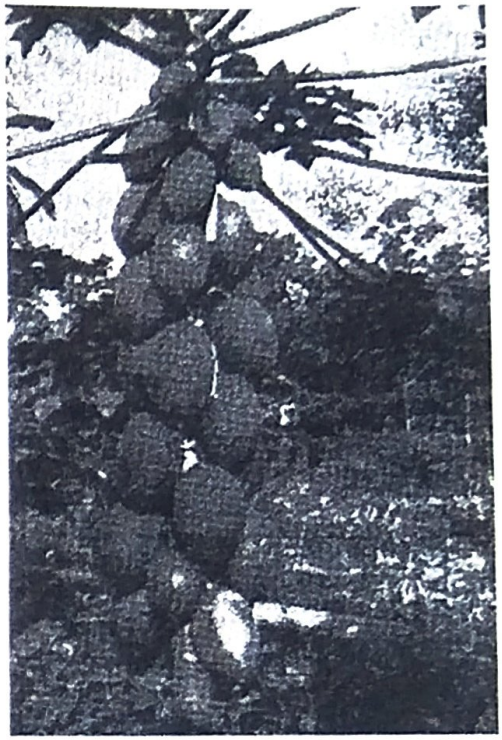

Fig. 8. C.P.L.-Dwarf and high yielding selection.

Ayappa and Nanjappa (1959) in Coorg selected Coorg Honey which is gynodioecious in nature. Two selections namely, CO1 and CO2 have been developed at Coimbatore by inbreeding of the cv. Ranchi for seven generations. Majumder (1980) selected dwarf, high yielding plant type (C.P.L.) with low fruiting height (Fig. 8) in Nigeria from Homestead papaya. The selection has dominant purple colour in all plant parts and serves as a marker gene.

At the IARI Regional Station, Pusa, separate lines of gynodioecious and dioecious from the cv. Ranchi have been developed. These have been released as varieties under the names Pusa Delicious, Pusa Majesty, Pusa Giant and Pusa Dwarf. Pusa Delicious and Pusa Majesty are gynodioecious while the other two are dioecious (Ram, 1981; 1982).

Heterosis breeding

Hamilton (1954) made elaborate selfing and crossing using dioecious and gynodioecious lines but did not observe either hybrid vigour or in breeding depression. Dai (I960) first reported heterosis in the cross between varieties 'Philippine' and 'Solo'. A high positive heterosis for fruit size and number of seeds was observed by Shah and Shanmugavelu (1975) in the cross CO1 × Coorg Honey at Coimbatore.

Iyer and Subramanium (1981) reported heterosis for all the vegetative characters, fruit yield and its components. Heterosis up to 111.41 per cent for yield was obtained in crosses Solo Yellow × Washington. Ram (1982a) has recorded marked heterotic response in crosses between Ramnagar Local and Washington and Pusa 1-15 × Halflong. No planned hybrid has reached a varietal status, however.

Mutation breeding

Ram and Majumder (1981) for the first time evolved dwarf mutant by treating seeds of papaya with 15 kR of gamma rays. Initially, three dwarf plants were

isolated from M_3 population. Repeated sib-mating among the dwarf plants helped in establishing a homozygous dwarf line which has been named "Pusa Nanha", and this has short petiole length and is eminently suitable for high density planting.

Breeding behaviour of inter-specific crosses

Of the 40 species of *Carica*, only a few have been utilised in breeding. These are *C. papaya, C. microcarpa, C. candamarcensis, C. cauliflora, C. monoica* and *C. goudotiana*. On the basis of crossability, these species can be arranged in three groups: (I) *C. monoica, C. cauliflora, C. microcarpa* and *C. candamarcensis*. These four species can be crossed readily with each other producing viable seeds (Jimenez and Horovitz, 1958); (II) *C. papaya;* and (III) *C. goudotiana*. Crosses between species of group I and II do not form mature seeds but in most cases the immature embryo can be cultured. Crosses between group II and III always give negative results.

Malaguti *et al*. (1957) on the basis of tests of mechanical transmission of papaya mosaic to different species of *Carica* have reported that *C. papaya, C. monoica* and *C. microcarpa* are susceptible while *C. cauliflora* and *C. candamarcensis* are resistant to mosaic disease. Capoor and Verma (1961) found *C. cauliflora* to be resistant to mosaic virus. Horovitz and Jimenez (1967) found that *C. cauliflora, C. pubescence, C. stipulata* and *C. candicans* are resistant to distortion ring spot virus.

Zerpa (1959) has made inter-specific hybrids of *C. cauliflora* × *C. microcarpa, C. monoica* × *C. cauliflora, C. monoica* × *C. candamarcensis,* F_1 (*C. monoica* × *C. cauliflora)* × *C. candamarcensis* and *C. cauliflora* × C. *candamarcensis* for cytological studies. Only the cross *C. cauliflora* × *C. candamarcensis* forms multivalents, all others form bivalents at Metaphase I. This indicates high interspecific genetic affinity.

Inheritance pattern

Hofmeyr (1938; 1939 and 1942) made detailed investigations of the genetics of some qualitative characters. He assigned the following gene symbols.

a — albino plant recessive to normal green;

d — dwarf plant recessive to normal tall plant;

dp — diminutive plant (short slender trunk, small leaves) recessive to large plant;

cp — crimpled leaf recessive to normal leaf;

rg — rugose leaf recessive to normal smooth leaf;

w — waxy leaf recessive to normal flat bladed leaf;

r —red fleshed fruit recessive to yellow flesh;

B —grey seed coat colour dominant to black seed coat colour.

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Mango

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12

Mutation Breeding

R.P. Sharma and V.L. Chopra

Since genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central to crop breeding. Experimentally induced mutations provide an important source of variability. An advantage of such created variability is that the starting point can be an agronomically accepted cultivar rather than a genetic stock of no direct commercial value with many undesirable attributes. It should, however, be realized that none of the imposed treatments in a plant system has so far been able to direct a specific change. As a result, following mutagenic treatments, a mixed bag of induced variants is found and many of these may not be of any value. Also, many events take place concurrently in a treated cell to produce variation at several loci within a genome. Because of these reasons it will be unrealistic to expect miracles out of a mutation breeding programme. The desired results can be achieved only when the objectives are clearly defined and the experiments are specifically designed to achieve those objectives. When a mutant of the required type is obtained, we only reach the step of identification of a desired recombinant in a conventional breeding programme and all the succeeding steps of evaluation of the mutant are in no way different from those in the conventional breeding procedures.

It will be relevant to first consider situations in which a mutation breeding programme can be recommended in addition to the recombination breeding programme. The following suggestions for recommended situations will be helpful:

1) When the natural variability does not provide the gene/s for the desired trait.
2) Even if the desired gene is available in a genetic stock, its known tight linkage to undesirable genes, would recommend the use of mutation breeding.

3) When a simply inherited defect in an otherwise superior agronomic cultivar needs to be rectified, induced mutagenesis provides the advantage of rectification without too drastic a change in the genetic background.
4) When the desired variation is required in a vegetatively propagated material.
5) In horticultural crops, particularly fruit trees, the consumer requirement of strict conformity to acceptable taste makes mutation breeding a method of choice for inducing changes while still retaining the desired attributes of consumer preference. For example, in apples a change from normal colour and flavour will hardly be acceptable even when a new variety is more productive and possesses other desirable characteristics.
6) When it is desired to introduce blocks at specific stages of a biochemical pathway to make alteration in the chemical composition in the produce of economic value, induced mutations will have a distinct advantage. In opium, the synthesis of morphine proceeds stepwise. Blocking the pathway at the bane level will prevent the synthesis of the narcotically active morphine without any disadvantage to the conversion process of the bane into the desired pharmaceutically active product.

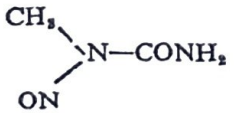
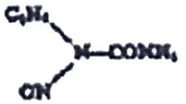
Choice of material

In crops propagated by seeds, treatment of the seed in dormant state is the routine procedure. However, treatment to seeds soaked in water and at a stage corresponding to DNA synthesis is the ideal target material for imposing mutagenic treatment. In the case of vegetatively propagated crops, the treatment can be given to cuttings, bud wood, suckers, or the tissue normally used for propagation.

Choice of mutagen

Since the discovery of mutagenic effects of ionizing radiations (Muller, 1927; Stadler 1928) and chemical compounds (Auerbach and Robson, 1947) the list of mutagens has become so large that a beginner is often confused about how to make a choice of the mutagen. The difficulties are compounded by reports attributing extraordinary efficacy to a particular mutagen in a particular system or a specificity of action to certain compounds. The first of these attributions is now borne out by a large body of data while the validity of the second still needs to be established. A safe recommendation would be the use of chemical mutagens like Ethyl methanesulphonate and some nitrosoureas (Nitrosomethyl urea) which have produced high mutation frequencies in a large number of plant systems. The inclusion of an ionizing radiation treatment, if the scale of the experiment permits, would also be useful on account of the total randomness of action of radiations on genetic material as also the fact that at optimal doses

Table 1. Commonly used chemical mutagens in plants

Name	Formula	Nature	Concentrations used Generally	Source
Ethyl methanesulphonate (EMS)	$CH_3SO_2OC_2H_5$	Colourless	0.1 to 0.3%	Kodak, Rochester, U.S.A. Koch-Light, Coinbrook (Bucks), England — Fluka, Buchs, Switzerland — Aristo custom chemicals Inc. 3813 Hires Lane Cincinnati, Ohio-45212 U.S.A.
N-methyl-N-nitrosourea (MNH)		Yellow solid	0.01 to 0.03%	
N-ethyl-N-nitrosourea (ENH)		Yellow solid	0.01 to 0.03%	-do-
Sodium azide	N_aN_3	White solid	0.001 to 0.004 M	E. Merck

radiations produce effects both through gene mutations and chromosomal mutations. Table 1 gives a list of only some of the mutagens which have given good results with crop systems and which are currently in common use. For more details on these and other mutagens, reference can be made to the Manual on Mutation Breeding, I.A.E.A. (1977).

Mode of action of the mutagens

1) Ionizing radiations

The most commonly used ionizing radiation in plant improvement programmes is gamma rays. Gamma rays are obtained from radioactive isotopes, like Cobalt-60 or Caesium-137. Because of their short wave length and high energy per photon, they have high penetrance. Table 2 gives the physical properties of ^{60}Co and ^{137}Cs and gamma rays obtained from these sources.

Table 2: Physical parameter comparisons of Cobalt-60 and Caesium-37

Physical parameters	Cobalt-60 (^{60}Co)	Caesium-137 (^{137}Cs)
Half-life	5.3 years	30 years
Wave length	0.1 Å	0.1 Å
Energy	γ_1 = 1.33 MeV γ_2 = 1.17 MeV	γ_1 = 0.66 MeV
Specific gamma ray emission	1.29 R (Roentgenllh. Ci (Curie) at a distance of one meter	0.3 R/h Ci at a distance of one meter
Specific activity	1-400 Ci/g	1–25 Ci/g
Shielding required	~5 cm thick lead	2 cm thick lead
Chemical form	Metal	Chloride or sulphate
β-emission	0.31 MeV (100%)	0.51 MeV (92%) 1.I8 Mev (80%)
Mode of production	$^{59}_{27}Co(n,r)^{60}_{27}Co$	Chemically separated fission product
Decay product	$^{60}_{28}Ni$	$^{137}_{56}Ba$

Source: Manual on Mutation Breeding, I.A.E.A., Vienna, 1977.

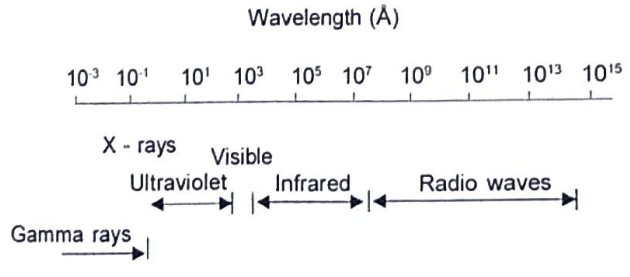


Fig. 1. Electromagnetic types of radiations and their wave lengths. *1Å = 10^{-8} cm

Gamma rays are electromagnetic type of radiations (Fig. 1) that induce ionization. When biological material is irradiated, a gamma ray photon hits an orbital electron of the atom. All the photon energy is transferred to the electron in the form of kinetic energy and the electron is ejected, leaving behind a positively charged atom. The ejected electron, also known as photo-electron, has tremendous energy and is capable of causing further ionizations along its path.

The transfer of energy by ionizing radiations is random but discrete. That is why, even a small amount of energy deposited by radiations produces ionization. It has been estimated that, on an average, every 32.5 electron volt (ev) of energy transferred induces one ionization. Heat energy, even of much higher quantum than radiation, does not produce any conspicuous biological damage because unlike radiation energy which is deposited discretely, heat energy gets uniformly distributed throughout in the atom.

Radiation induced biological damage is brought about in two ways: (i) direct effect—where DNA is the direct target and radiation energy is directly absorbed by it, and (ii) indirect effect—where the initial absorption of energy is by other molecules in the cell. Subsequently this energy or the products of this energy (highly reactive radicals) can be transferred to DNA. Under normal situations, it is difficult to separate these effects and therefore, the biological effects observed following ionizing radiations are the cumulative effect of both direct and indirect effects of radiation.

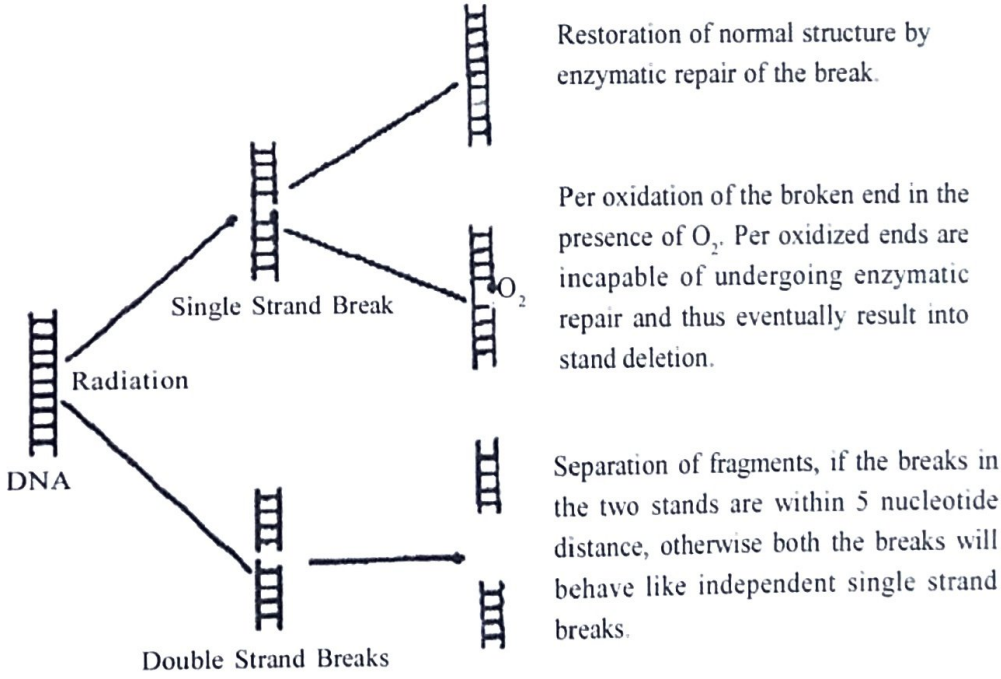

Restoration of normal structure by enzymatic repair of the break.
Per oxidation of the broken end in the presence of O_2. Per oxidized ends are incapable of undergoing enzymatic repair and thus eventually result into stand deletion.
Separation of fragments, if the breaks in the two stands are within 5 nucleotide distance, otherwise both the breaks will behave like independent single strand breaks.
O_2
Single Strand Break
Radiation
DNA
Double Strand Breaks

Fig. 2. Single and double strand breaks in DNA following treatment with ionizing radiations.

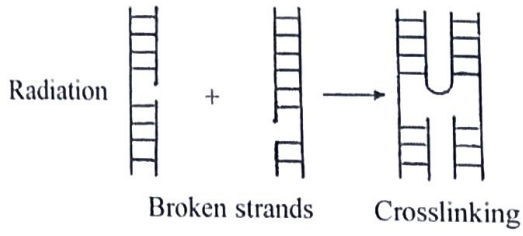


Fig. 3. Crosslinking between two DNA molecules.

Exposure to radiation energy

Discontinuity in DNA

Enlargement in the gap by enzymatic activity

Repair of the gap; if repair is faulty, mutation occurs by base alteration

Fig. 4. Schematic diagram of mutation induction from ionizing radiation exposure.

The major effects of ionizing radiations on DNA are: disruption of continuity of one or both strands (Fig. 2, c.f. Bacq and Alexander, 1961), cross-linking of DNA-DNA (Fig. 3) and DNA-proteins, removal of a base and chemical alteration of a base which changes its pairing properties (c.f. Casarett, 1968). While cross-linking of DNA leads to cell death, the strand breaks result in chromosomal mutations and the repair of radiation induced nicks in DNA (Fig. 4) and base changes in gene mutations.

2) Chemical mutations

As mentioned earlier, the list of available chemical mutagens is very large. However, relatively limited number will suffice for use for mutation breeding purposes in crop plants as has been demonstrated by their actual use. The preferred mutagens belong to the class of alkylating agents (Ethyl methanesulphonate, Nitro-methyl urea, Nitrosoethyl urea). Alkylation refers to the substitution of an alkyl group (*e.g.* C_2H_5 of EMS) for a hydrogen in the nitrogenous bases. The alkylation of DNA leads to the following effects.

i) Alkylation of the phosphate groups of DNA: Alkylation leads to the formation of phosphate triesters which are unstable and release the alkyl group. However, if enough alkyl groups remain unreleased, then the attached alkyl groups interfere with DNA duplication. Sometimes the phosphate triester is hydrolyzed between the sugar and the phosphate and results in breakage of the DNA backbone.

ii) Alkylation of the bases: The seventh position in the guanine is a preferred site for alkylation but it has been established that the major mutagenic effects arise from O^6 alkylation of guanine. O^6 alkyl-guanine can pair with thymine and leads to base pair transition (Fig. 5).

iii) Depurination: The alkylated guanine can separate from the deoxyribose leaving it depurinated. The gap can be filled up by any base during DNA duplication leading to transversions or transition type of mutations (Fig. 6).

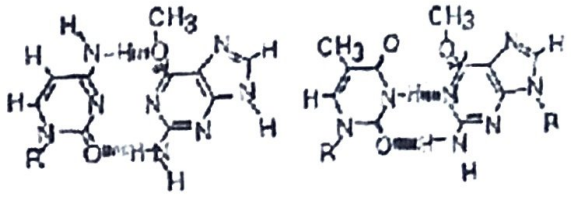


Fig. 5. Potential base-pairing of O^6-methylguanine with thymine and cytosine. Note that the rotation of the O^6-methyl group (indicated by arrow) can cause steric hindrance in H-bonding with N-4 of cytosine.

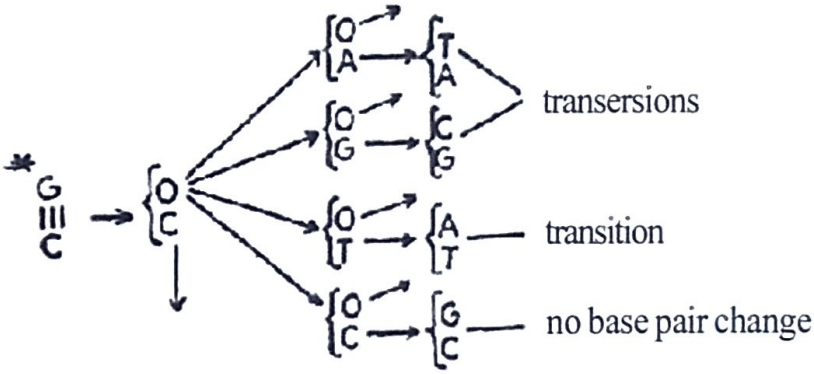


Fig. 6. The gap (O) created by the removal of alkylated guanine (*G) from DNA can lead to different types of base pair replacements.

Mutagen dose

One of the most crucial requirements for a successful mutation breeding programme is the selection of an effective and efficient dose of mutagen for mutagenizing the starting material. Historically, effectiveness of a mutagen has been measured in terms of the biological effect that it produces. Judging by this criterion a mutagen that produces a high degree of physiological and chromosomal effect is said to be more effective. From the point of view of mutations induction, however, the induction of heritable changes by a mutagen is the important criterion of its efficiency. A biologically more effective mutagen, therefore, need not necessarily be efficient in mutation induction. A distinction between effectiveness and efficiency of mutagens has been a major experimental activity in the past.

The purpose of this exercise was to identify criteria by which efficient mutagens and mutagenic doses can be selected on the basis of analysis of M_1 parameters (Konzak *et al.*, 1965). A generalized conclusion from years of fertile academic activity was that none of the M_1 parameters reflects mutagen efficiency as a uniform criterion. This is not entirely unexpected because induced mutations are manifestations of complex biological events which are profoundly influenced by numerous factors like genotype of the treated organism and post mutagenic treatment conditions.

When confronted with a situation where existent published information is not available, resorting to use of LD_{50} or GR_{50} is often used. With ionizing radiations, a dose which restricts survival to 50 per cent (LD_{50}) or growth to 50 per cent (GR_{50}) is a good treatment (Table 3). In actual practice one uses two to three doses around the LD_{50} dose. In the case of chemical mutagens, where the determination of LD_{50} is complicated by the difficulty of determining effective termination of treatment, the practical way is to use a graded series of dilutions or treatment duration on the basis of reports in published literature (Table 1). As a way of reducing the number of treatments, and the consequent large amount of material that will have to be screened in later generations, a dose and treatment regime can be worked out which reduces the growth attained in a fixed period to 50 per cent of the control (GR_{50}). Actual treatments then can be: (i) a dose that reduces growth to 50 per cent, (ii) a concentration above it, and (iii) one below it.

Size of the mutagenized population

To ensure a reasonable chance of the recovery of the desired kind of mutants, the surviving M_1 population is of critical importance. Making up the shortage of M_1 by enlarging the M_2 population is no solution. The ideal size of M_1, on the other hand, will depend upon the nature of inheritance of the gene/s for the character under consideration as well as the expected mutation rate. For example, for a character under single gene control, the required M_1 population to raise the M_2 is expected to be smaller than if the character is due to the interaction of two or three genes. For polygenically controlled traits, where the basic aim of induced mutagenesis is to enlarge variability, the needed M_1 population can be comparatively small because the number of loci at any of which a change will be effective is much larger. Brock (1971) has suggested a method for estimating the number of M_2 families that should be examined for a reasonable chance of isolating mutations of different kinds that occur with varying frequencies. The components of the computing formula are given below and the information derived from it is presented in Table 4.

Table 3. Gamma ray doses needed for 50 per cent growth reduction (GR_{50}) in some of the plant families

Families*	Dose Range (K rad) for GR_{60}
Gramineae	20–40
Solanaceae	30–60
Cruciferae (*Brassica oleracea*)	120–140
Liliaceae *(Allium cepa)*	20–30
Cucurbitaceae	35–60
Leguminosae	04–100
Vicia faba major	4–6
Pisum sativum	10–27
Cicer arietinum	18–26
Phaseolus vulgaris	15–30
Arachis hypogaea	35–45
Phaseolus aureus	65–100

*To a great extent radiosensitivity depends on the genotypic make up of an individual, thus species and even strain differences for GR_{50} dose are expected. For accurate estimation of LD_{50} or GR_{50} a number of doses within the dose range given should be tried.

Source: Abridged from Table V on Radiosensitivity of a number of crop species to gamma and fast neutron irradiation in *Manual on Mutation Breeding*, I.A.E.A., Vienna, 1977, p. 44

$$n = \frac{\log (1 - p_1)}{\log (1 - \mu)}$$

where

n = number of M_2 families
(μ = mutation rate
p_1 = probability for the occurrence of at least one mutation

Table 4: Number of M_2 families to be examined for various mutation rates and probabilities of occurrence

Mutation frequency (μ)	Number of M_2 families (n)		Type of mutation
	$P_1 = 0.90$	$P_1 = 0.99$	
1×10^{-2}	233	465	Chromosome changes and quantitatively inherited variability
1×10^{-3}	2,326	4,652	Several recessive genes
1×10^{-4}	23,260	46.520	Single recessive gene
1×10^{-5}	232,600	465,200	Single dominant gene

The number of M_2 plants per M_2 family can be calculated from the formula:

$$m = \frac{\log (1 - p_2)}{\log (1 - a)}$$

where

m = number of M_2 plants per family

a = segregation ratio of the mutation

p_2= probability of occurrence of at least one homozygous mutant

The segregation ratio of a mutation controlled by a single recessive gene in M_2 is 1/4 following mutagenic treatment to a haploid gamete (pollen). However, when seeds are mutagenized, it is generally more than one cell of the treated embryo that contribute to the next generation. Under these situations, the segregation ratio of the mutation is changed. The data presented in Table 5 gives the sizes of the M_2 families for different segregation ratios.

Table 5. M_2 family sizes for different segregation ratios and levels of probability of occurrence of the homozygous mutant

Segregation ratio	M_2 family size (m)	
	$P_2 = 0.90$	$P_2 = 0.99$
a = 1/4	8.0	16.0
1/8	17.2	34.5
1/12	26.3	52.6
1/16	35.7	71.4
1/20	45.5	91.0

Combining the above two estimates, that is, the value of V and 'm', the population size required in M_2 for isolating a given type of mutation with a given probability can be calculated. Thus, for a mutation controlled by a single recessive gene and having mutation rate equivalent to 1×10^{-3}, the M_2 population to be examined is approximately 50,000. If the mutation frequency for a trait is 1×10^{-5} then the M_2 population needed to be analysed will be 5,000,000.

The population size given in Table 4 is optimum for successfully recovering a given type of mutation. In practice, however land and labour constraints generally do not permit raising such a large population. Nevertheless, it should be borne in mind that in order to achieve the desired results with certain amount of certainty, there is no other alternative but to grow as large a population as is practicable.

Handling of M_1 generation

The mutagenized material is normally sown directly in the field. For seed material which already contain several primordia at the time of treatment, like those in wheat and barley, a close planting is recommended to cut down the number of tillers. This is done because the frequency of induced mutations is higher in the primary as compared to late order tillers. In experiments where inferences are to be drawn on mutation frequencies, genetic purity of the material treated for raising the M_1 is important. It is also important to rigidly control pollination in the M_1 to prevent outcrossing. Monoceous plants, like maize, present the peculiar situation that the androecium and gynoecium develop sequentially and have considerable developmental time lag. The two, because of their development from different tissues, may not share the same mutation. Therefore, even if selfed, they will generate some heterozygotes. It has been suggested by Singleton (1969) that, in such cases, the M_1 may be allowed to intermate and selfing should be done only in the M_2. The scoring of mutations therefore will be done in M_3.

The harvesting of the close planted M_1 is recommended plantwise. In the case of cereals, where more than one effective tillers are produced, sometimes the identity of the tillers, in terms of sequence of their appearance is also maintained for the purpose of comparing mutation frequencies in the tillers in relation to their developmental sequence. In the case of monoceous plant species, the M_1 can be harvested as bulk.

Parameters on which reliable genetic data can be recorded in M_1 are: dominant mutations, recessive chlorophyll sectoring, and seed sterility. These data, for meaningful interpretation, must be supported by observations on M_2.

Observations on M_2 generation

The M_2 is raised as single plant, or single head progenies and is suitable for scoring single gene recessive mutations for chlorophyll, morphological, disease resistance, biochemical (auxotrophs) and other qualitative traits. When scoring for quantitative characters is done in M_2 itself, it is necessary to raise M_2 plants at equal spacing. Single plant or progeny selection in M_2 on visual criteria is not valid; quantitative data on the character of interest is essential for this purpose. Quantitatively inherited characters also require special considerations, in view of the fact that restriction of material that is to be carried forward to the next generation, on a rational basis, assume critical importance.

Several procedures of handling mutagenized population for selection of quantitatively inherited traits have been advocated and each of them has its merits. In the following, a procedure is given which recommends itself on the

basis of theoretical considerations and proven successful practical application. The advantages of the procedure arise from: (i) a large starting mutagenized population, (ii) statistical approach for advancing all possible genotypes on family basis from M_2 to M_3, and (iii) making individual plant selections, again based on biometrical parameters in M_3, a generation when certain amount of homozygosity has been attained. The procedure operates in the following steps:

1) Raise a large M_1 by close planting the mutagenized seeds.
2) Protect the M_1 plants from cross pollination.
3) Harvest each M_1 plant separately.
4) Raise M_2 from each M_1 plant by providing equal spacing between plants.
5) Take observations on at least five plants looking normal for the trait to be improved (including yielding and quality) and calculate means and variances.
6) Preferably select those M_2 families which show high mean and high variance; alternatively select families with normal mean and high variance.
7) Bulk each selected M_2 family separately.
8) From each selected M_2 family, raise sufficient population in M_3 so that it is representative of genotypic frequencies of the family.
9) Take observations on all the M_3 plants and select individual plants conforming to selection needs. In practice, this is done by selecting plants having a higher mean for the desired attribute.
10) Raise M_4 from each selected M_3 plant in a rod-row trial for preliminary evaluation. Use the best available genotypes as the check.
11) Select the promising lines in M_4 and evaluate in M_5 in station trials.
12) Enter the best lines in coordinated trials for evaluation and possible release.

Factors affecting realization of mutations

A mutational event at the DNA level need not necessarily be observed as a mutant plant because a series of events intervene between the reaction of a mutagen with DNA and the realization of the mutant. The mutational lesion has to pass through the following sieves before the mutant expresses as a phenotype.

i) Repair: Some of the mutational lesions in DNA are enzymatically repaired to restore the original condition and therefore these mutational events go unscored.

ii) Diplontic selection: The mutated cell is often at a competitive disadvantage compared to the unaffected normal cells. Because of this, the normal cells proliferate much more rapidly and crowd out the mutant cell. At times the sector of mutant cells is so small that it may be excluded from contributing to

the formation of gametes. This exclusion of the mutant cells by the normal cells is called diplontic selection.

iii) Haplontic selection: When the competitive disadvantage operates at the gametic level, the functioning of normal gametes leads to haplontic selection. The causes of the poor competitive ability of the mutant gamete could be developmental derangement, lack of dispersal, poor germinability or physiological malfunctioning.

iv) Mutant expression: The phenotypic expression of a mutant allele is, like all genetic characteristics, strongly influenced by environment. The requirements for proper expression of a mutant may be distinctly different from the requirements of the parental allele and therefore, the mutant phenotype may not develop in a plant grown under the environmental conditions in which the mutagenized variety is normally cultivated.

Handling mutagenized populations for specific breeding objectives

1) Mutants for yield

Yield is a complex character. The yield controlling genes have three peculiarities which must be considered while applying the induced mutagenesis pathway for affecting improvement. These are: (i) the inheritance of yield is quantitative, (ii) there is a strong environmental influence on the expression of yield genes, and (iii) the environment that a mutant might need for the best expression of yield may not be identical with the requirements of the parental variety.

Two procedures have been employed for identifying high yield mutants:

The first procedure involves manipulation of the components of yield (Gustafsson 1965; Swaminathan, 1965; Sharma, 1982). The requirement is best tackled when the yield parameters are identified and well defined and their genetics are known. The procedure has the advantage that individual mutants need not necessarily have a simultaneous change in several characteristics that go to make good yield but their similarity of genetic background allows combination of characteristics by intercrossing of mutants. It is also known that some components, like test weight in wheat, have a high heritability and selection of mutants affecting such characteristics is relatively easy and more reliable.

The second alternative directly assesses yield as an end point (Gregory, 1956; Sigburbjornsson and Micke, 1974). For this purpose, mutants are identified according to the procedure outlined above for isolation of quantitative characters. Standard evaluation procedures, as applicable to the recombination breeding techniques, are applied for processing the material for possible commercial release.

2) Growth and reproductive characteristics

Present day demands of intensive agriculture require different combinations of growth and reproductive characteristics so that the varieties can fit into different cropping systems. Genetic dwarfing is a growth characteristic which confers many advantages, such as lodging resistance, ability to utilize high inputs of fertilizers and water and higher harvest index. Induced mutagenesis has been an efficient method of obtaining short statured genotypes in acceptable genetic background (Gustafsson *et al.*, 1960). Also important are compact growth habits for intercropping (as in pigeon pea), erect growth habit (as in chickpea), suitable leaf disposition angle for better inter-ception of incident light in cereals and branching pattern in mustard. Among the reproductive traits, the flowering time becomes critical in several situations. Synchrony of flowering, and the consequent synchrony in development of the sink, is another characteristic of advantage. For both these characteristics mutants have been induced and exploited, for example Aruna is an early mutant of castor bean (Kulkarni, 1969). Aruna flowers in 120 days as compared to 270 days for the mother variety, and is also superior in yield.

3) Breeding for quality

While yield is the most important breeding objective, due emphasis is necessary for quality attributes because on them depends the acceptability of the improved cultivar by the consumer. For different crops, the attributes of quality differ widely. In cereals and pulses, the protein content and the amino acid balance of the proteins are important characteristics. In the case of oilseeds, fatty acid composition, oil extractability and clearance and storability of oil assume importance. Certain seeds, particularly those of legumes, contain anti-nutritional factors. In fodder crops like sorghum, the hydrocyanic acid content is important in determining the fodder quality.

Breeding for quality is complicated because it has necessarily to be combined with yield and its identification in the segregating population poses a problem. It is because of this reason that, traditionally, the major emphasis in breeding has been placed on yield and the improved lines are then tested for quality characteristics. It is now realized that this is not the ideal procedure because the end point testing provides no mechanism for its manipulation other than starting all over again. Currently, it is recommended that incorporation of quality characteristics should be integrated with the normal breeding procedures for yield. While advancing the improved material, yield and quality should be concurrently considered together. When so practiced, quality becomes just another attribute for which selection is exercised as operative for any other character.

4) Disease and pest resistance

Developing varieties and genetic stocks resistant to diseases is a particularly important area in which induced mutagenesis can be profitably exploited. There are several reasons for this situation. The important among these are:

a) Disease resistance has great evolutionary advantage in nature. Therefore, resistance is more often found in the wild forms. Experience has shown that transfer of resistance invariably brings along linked genes for low productivity traits, even when the resistance occurs in the same species. When the resistance is to be derived from a wild relative, removed by specific and even generic taxa, the problems of transfer become even greater. Induced mutagenesis provides a way out by creating variants which can be directly developed into varieties or can constitute usable genetic stocks.

b) The screening procedures which can be imposed on segregating material, make identification of a mutant easy. Large populations can be easily screened.

c) In crop species where the breeding efforts have brought the productivity levels to a plateau, induced mutants for disease resistance have a better chance of becoming acceptable varieties, because the resistant mutants will not have to compete in yield performance with newer strains developed specifically for yield.

d) Mutational approach can be effectively used for developing multilines in a much shorter time compared with the conventional backcross procedures. Since simultaneous mutations have a reduced probability of occurrence, a mutant with race-specific resistance is not likely to be accompanied by a sizable derangement of the genetic background. Mutants resistant to different races or race combinations can therefore be compounded to develop a multiline.

e) In species lacking a sexual cycle, induced mutagenesis for resistance offers the sole practicable procedure of incorporating genetic resistance.

f) In the extreme situations where a gene for resistance against a disease is not available in the existing germplasm or is too rare to locate in the wild, induced mutation for resistance is the only available alternative.

Because of the advantages briefly outlined, considerable efforts have gone into isolating mutants resistant to diverse diseases in a variety of crop plants (IAEA 1977; Micke, 1983).

Experience of a large number of mutation workers in the disease-resistance area has brought another interesting point into focus. This relates to the facility

with which resistant mutants can be isolated. Part of this case can be attributed to the availability of procedures that are very effective for screening the mutagenized populations. Another contributory factor is the large number of loci at which a change could occur resulting in resistance. This is illustrated by the large number of genes that have been identified to confer resistance against physiological specializations of disease causing organisms (Potato late blight; Linum flex rust; Wheat rust; etc). In spite of these advantages, the scale of most reported experiments on mutagenesis for disease resistance should have precluded the possibilities of success. In actual practice, however, it is found that resistant mutants have been obtained in experiments of fairly modest dimensions. While an easier explanation would be to attribute it to outcrossing promoted by the concomitantly induced sterility in M_1, the possibility should also be considered (Chopra *et al.*, 1983) that the resistant mutants may occur with frequencies much higher than those expected on theoretical calculations reported by Brock (1971).

5) Stress tolerance

Breeding for tolerance to various kinds of stresses assumes importance in agriculture because the proportion of stressed agriculture, even though variable, is large in absolute magnitude. In the traditional breeding approach, success of incorporating stress resistance has been small because of a lack of understanding of the parameters that contribute to stress resistance. The mechanism by which such resistance operates is yet far from clear. Success has also been hindered because of the difficulty of artificially creating the relevant stress under field conditions. Most attempts towards breeding for stress tolerance have been based on empirical procedures. Some workers, however, have taken recourse to screening of naturally occurring and induced variability for resistance to certain stresses like excessive salt or high concentrations of certain elements, by growing plants in liquid cultures with appropriate additives (Nieman and Shannon, 1976; Patil and Sharma, 1985). Employing such procedures, varieties resistant to salt in barley crops have been developed and released (Epstein, 1976).

With the development of the tissue culture technology for crop plants, it is being hoped that selection of natural variability as well as of induced mutations at cellular level will become possible under laboratory conditions.

Induction of mutations in vegetatively propagated plants

Vegetatively propagated plants require special considerations both in the choice of the mutagen used as well as handling of the mutagenized material.

Choice of mutagen

The vegetative tissue, which is the target of mutagenic treatment, is usually a multicellular differentiated bud and poses problems of penetrance when treatment is given with a chemical mutagen. Ionizing radiations are therefore preferred mutagens for vegetatively propagated plant species. In cases where treatment with chemical mutagens is desired, penetrance can be enhanced by dissolving the mutagen in solvents like dimethyl sulphoxide (DMSO).

Mutagen dose

Mutagenic doses, close to LD_{50} or GR_{50} dose, are generally recommended.

Handling of mutagenized material

As already mentioned, buds which are subjected to mutagenic treatments, are multicellular and therefore induced mutations occur only as sectors. Under these conditions, the forces of diplontic selection influence greatly the realization of mutations.

It is now generally accepted that the shoot apex, axillary buds and adventitious buds contain three independent germ layers, L-I, L-II and L-III (Fig. 7), which are also referred to as histogenic layers. These layers eventually give rise to a fully differentiated shoot with leaves and axillary buds.

Following mutagenic treatment, any cell/s in the three histogenic layers can get mutated. The position of the mutated cell is, however, important for its further fate. If the mutated cell and its lineage give rise to a leaf then it is rather difficult to recover mutations from such cells. However, if the mutant cell contributes to the formation of shoot (Fig. 8) and results in mericlinal chimera (Fig. 9) then either by repeated cut-backs (in case of woody plants, Scheme 1A) or by raising successive generations (in case of tubers, Scheme IB), chances of axillary bud developing from the mutated tissue and giving rise to a mutated shoot can be increased (Fig. 10).

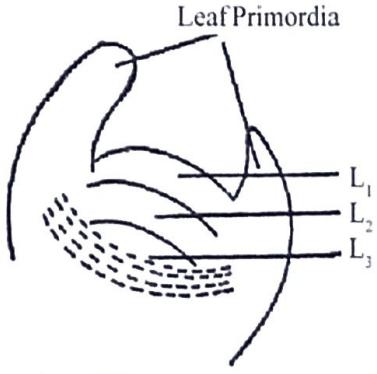


Fig. 7. Three histogenic layers in a differentiated shoot apex. Axillary buds and adventitious buds also show similar developmental pattern. Dotted area denotes cytohistological zonation.

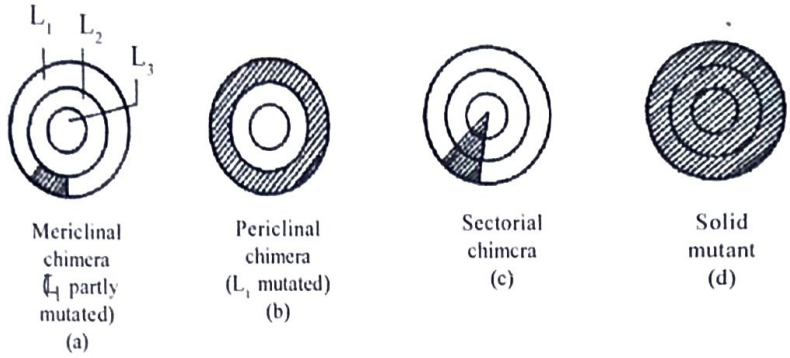


Fig. 8. Cross sections of shoot apices showing the position of mutated cells in diffe-rent types of chimeras. Mericlinal and periclinal chimeras shown in (a) and (b) for L_1 can involve any of the three histogenic layers. Rearrangements of cell layers is known to occur. This can lead to replacement of, for example L_1 by L_2 or L_3 layers and vice-versa. Thus, mericlinal in L_2 can eventually result into periclinal in L_1.

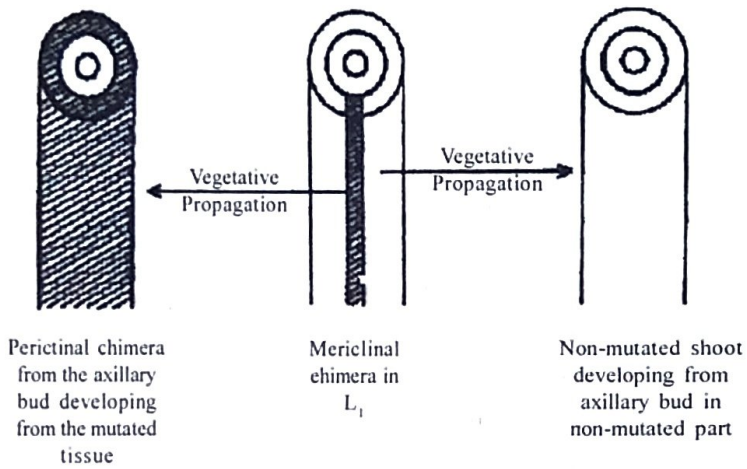

Vegetative Propagation
Vegetative Propagation
Perictinal chimera from the axillary bud developing from the mutated tissue
Mericlinal chimera in L_1
Non-mutated shoot developing from axillary bud in non-mutated part

Fig. 9. Position of axillary bud in a shoot, developing from mutagenized open or axillary bud, and realization of mutation.

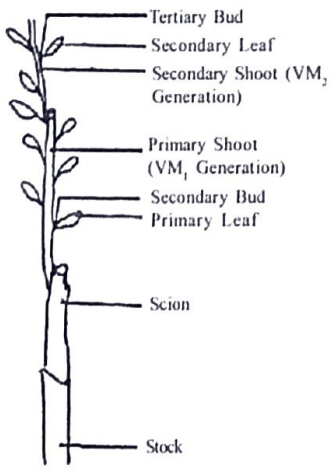


Fig. 10. Schematic presentation of irradiated scions and primary and secondary buds, shoots and leaves in apple.

Another method by which the forces of diplontic selection can be avoided is the treatment of adventitious buds. This technique makes use of the fact that adventitious buds formed at the base of the petiole of separated leaves originate from a single/few epidermal cells. More than 350 plant species can be propagated from adventitious plantlets on separated leaves. However, the success of this technique depends on intrinsic factors like age of the leaf and leaf part and extrinsic factors like rooting medium and cultural conditions (temperature, humidity, light etc.). It is, therefore, necessary to standardize these factors for the plant species to be used in mutation breeding.

Scheme 1

Schematic representation of steps involved in handling of mutagenized plant parts propagated through cuttings or buddings in—

A. *Woody species*

—Mutagenize plant part having normal or adventitious buds.

—Allow the bud to sprout (VM_1).

—Repeatedly prune (VM_2, VM_3.....etc.) the growing shoot to promote the cells from mutated sectors to regenerate (Fig. 10).

—Take cuttings from the mutant branch for multiplication.

—Evaluate for possible release.

*Each pruning cycle represents a vegetative cycle.

B. *Tuber crops*

—Mutagenize full tuber, half tuber or disks containing single eye.

—Raise VM_1.

—Select tubers showing sectorial chimera in VM_1.

—Raise VM_2.

—Select solid mutation in VM_2.

—Raise VM_3 to confirm breeding behaviour and multiplication of the mutation.

—Evaluate in successive generations for the desired attributes and possible release.

The contribution and relevance of mutation breeding

In the early years of mutation research much was expected of its potential for developing improved varieties of crop plants. In situations where the crop

improvement efforts were in their infancy and dramatic improvements were not being effected through conventional breeding, many examples appeared of mutants that were directly processed into varieties. Authentic information on the area covered under these varieties, however, unfortunately is lacking. It is accepted that Aruna, a mutant variety of castor, did cover sizable acreage on its release for cultivation in parts of India. Also, the Trombay Series of Groundnuts (TG numbers), because of their very bold kernels, have found wide acceptability as varieties for table purposes. In international agriculture, however, there are several examples of striking contributions of mutant varieties. Mutant variety Pallas of barley, for example, covered nearly 40 per cent of barley acreage in Denmark in 1966. The approximately $ 25 million peppermint industry, based on a single clone of *Mentha piperetta* variety "Dricham", in the U.S.A. was saved from a disaster by the mutant varieties "Todds Micham" (Murray 1971) and "Murray Micham" (Murray 1976) when the parental variety became susceptible to Verticillium wilt. In vegetatively propagated plants, and in ornamentals, induced mutation, even to this day remain a major source of new varieties.

In the overall context, however, it will be relevant to consider what direction should the mutational approach adopt for making continued contribution to crop improvement activity. In the Indian situation where all varieties, irrespective of the method through which they are developed, have to go through the coordinated grid of testing, mutational rectification of a defect will have limited use in crops where intensive breeding activity is in progress. This is because in the defect rectified variety, the yield potential is not expected to change and during the period of testing the mutant, varieties bred specifically for higher yield, would have come on the scene. It is for this reason that in the future mutation breeding is expected to make a contribution primarily as an important adjunct to the conventional breeding approach. Survey of recent literature amply indicates that major gains have resulted from the use of induced variability when it was fully integrated with crop breeding and more varieties have resulted by using induced mutants in crossing programme rather than processing them directly into varieties. A schematic outline of steps that will provide a viable procedure for integrating conventional (recombination) and mutation breeding is given in Fig. 11.

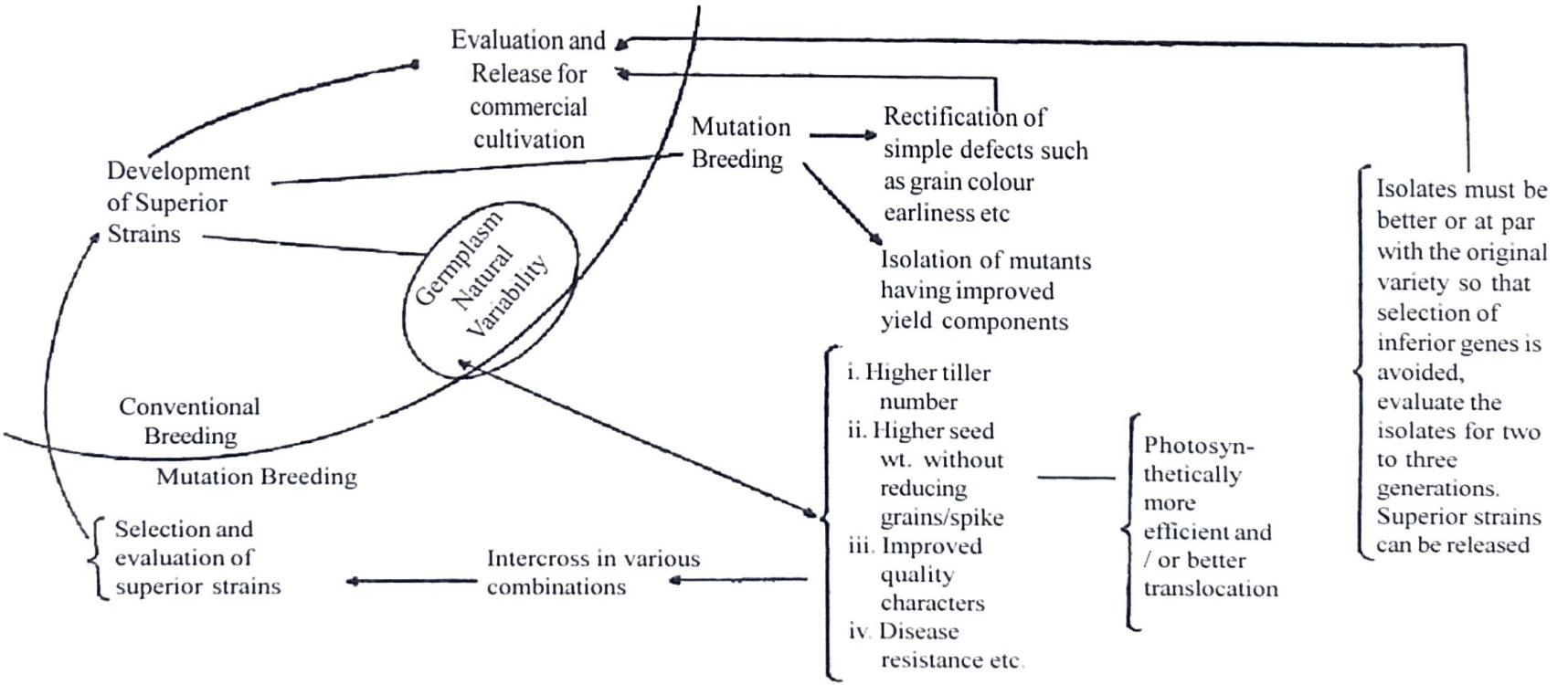


Fig. 11. Scheme for integration of conventional and mutation breeding with special reference to self-pollinated crops (adopted from Sharma and Sutar, 1977).

Table 6. List of plant cultivars derived from induced mutants or the use of induced mutants in cross breeding in India

	Plant species	*No. ofvarietie*
listedArachis hypogea	4	
Bougainvillea sp.	—	4
Brassica juncea	—	1
Cajanus cajan	—	1
Capsicum annum	—	1
Chrysanthemum sp»	—	30
Corchorus capsularis	—	1
Corchorus olltorius	—	1
Dahlia sp.	—	10
Gossypium sp.	—	3
Hordeum vulgare	—	12
Lens culinaris	—	1
Lycopersicon esculantum	—	2
Oryza sativa	—	8
Pennisetum americanum	—	2
Phaseolus vulgaris —		1
Pisum sativum	—	1
Polyantha tuberosa	—	2
Portulaca grandiflora	—	11
Ricinus communis	—	3
Rosa sp.	—	5
Saccharum officinarum	—	
Sesamum orientale	—	1
Sinapis alba	—	1
Solanum bhasianum	—	1
Triticum aestivum	—	4
Vigna radiata	—	2
Vigna unguiculata	—	4
		106

Source: International Atomic Energy Agency (1985) Mutation Breeding Review No.3 (May, 1985).

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8) Parents of hybrid varieties
9) Cytogenetic stocks/testers
10) Mutants

c) Genetic resource at molecular level

11) DNA libraries *in vitro*

1) *Related wild taxa*

Plant populations which originated and continue to grow under natural conditions, without any interference from mankind, are called wild forms or taxa. Genetic differentiation of such populations is mediated by natural mutations, hybridization and selection. The wild species are direct or indirect progenitors of all domesticated species that constitute crops. The magnitude of differentiation or genetic distance, between a crop species and its progenitor wild form is often related to the time span of domestication, extent of cultivation and degree of intervention by mankind. In several major crop species, such as rice, tetraploid cotton, tobacco, etc., it has been possible to locate link forms and trace the genetic transformations connecting wild progenitors and cultivated forms. In some other major crops like maize and cultivated hexaploid wheat, the ancestors have not clearly been identified. On the other hand, in several fruit, pasture, medicinal and agro-forestry crops, wild species constitute recent domesticates. Thus, there is an evolutionary continuum between wild forms and the crop derived from them, and the entire spectrum has a connecting genetic thread.

During the recent decades several wild species have been directly domesticated as commercial crop plants. Further, wild progenitors constitute important gene pools, especially for adaptation to stress conditions and resistance to pests and diseases, which could be shared by cultivated forms. In some cases, wild taxa are the only source of resistance to diseases threatening production of certain crops. For example, in rice, the foremost food crop of Asia, the only source of resistance against grassy stunt virus was found in the wild species *Oryza nivara* collected from Uttar Pradesh in India.

2) *Weedy forms*

Weedy forms are usually byproducts of domestication of wild forms and represent an intermediate step between wild populations and crops. Weedy forms usually evolve under disturbed habitats of cultivation or on borders of cultivated lands. Weeds have evolved in competition with crops and the two have often been introgressed by each other. Weeds generally have high fitness and possess unique adaptation traits.

3) *Land races*

"Land races", according to J.R. Harlan (1975) "have a certain genetic integrity They are recognizable morphologically; farmers have names for them and different land races are understood to differ in adaptation to soil type, time of seeding, date of maturity, height, nutritive value and other properties. Most important, they are genetically diverse. They are balanced populations—variable, in equilibrium with both environment and pathogens, and genetically dynamic..."

Land races have evolved under subsistence agriculture, possess genes for greater adaptability, durability of resistance and tolerance to adverse environments, but have low productivity. They however, respond to various ecological situations and selection pressures including selection for higher yields. When highly productive genotypes are evolved by breeding, the land races undergo rapid replacement. Since they possess valuable alleles, the land races should be conserved.

4) *Obsolete varieties*

Some varieties which were popular at one time for their special traits get replaced by high yielding varieties (HYV) later. For instance, in India wheat varieties K 68 and Pb 591 were most popular traditional tall varieties in the pre-HYV era. They had excellent grain and *chapati* making quality. But these varieties are almost out of cultivation. They are good genetic resources and have been used extensively in wheat breeding programmes in India and abroad particularly for grain quality.

5) Breeding lines with particular genes and performance

Certain varieties possess special traits and are grown for them though they may not be high-yielding. For instance, *Basmati* rice varieties, commonly grown in India and Pakistan, possess unique aroma and grain quality and fetch premium prices in the local as well as foreign markets. The maintenance of such genotypes as germplasm lines is important and requires special care.

6) *Prebreeding materials*

These are also varieties possessing special traits but usually obtained as recombinants from interspecific hybridization. They are potential sources for further improvement of the existing cultivars.

7) *Advanced cultivars*

Advance cultivars consist of improved, modern, high yielding varieties. They are well-represented in existing collections and constitute a major part of working collections. These resources are usually in greater demands from other countries,

but their exchange or sharing with other countries depends a great deal on the economic importance of the crop, especially its competitive value in international trade. For instance, while most of the countries exchange modern varieties of rice and wheat, the same countries have tight restrictions on sharing modern varieties of commercial plantation crops such as oil palm, pepper, cloves, cardamom and others.

8) *Parents of hybrid varieties*

Hybrid varieties (Fls) are becoming increasingly popular because of their high productivity and uniformity of produce. Most commercial hybrids are based on three-line breeding approach *i.e.* involvement of cytoplasmic male sterile (A) line, maintainer (B) line, and restorer (R) line. Cytoplasmic male sterility sources are usually found in wild species, and there is great variability in the cytoplasmic sources for causing male sterility, restoration interaction and side effects.

9) *Cytogenetic stocks/testers*

Cytogenetic stocks/testers such as aneuploids, translocation testers, haploids, isogenic lines, etc. are extremely valuable genetic resources for cytogenetic and genetic analyses and for transfer of specific chromosome segments or block of genes. Development as well as maintenance of such stocks is complicated and expensive.

10) *Mutants*

Induced as well as spontaneous mutants have greatly added to genetic diversity of many crops. They are potential sources for breeding for higher productivities.

11) *DNA libraries in vitro*

With the advances is molecular biology according to W.J. Peacock (1984), "the construction of new variability or novel genes, which may well be outside the available genetic resource material, is something that should be considered (as a possibility) and is likely to be an adjunct to any genetic resource activity." It should now be possible to isolate genes, mutate them and then reinsert them. Further, it may be possible to incorporate part of a plant virus genome into a plant genome such that its expression would lead to cross-protection against related virulent forms of the virus. Similar genetic engineering could be envisaged for pathogenic fungal or bacterial genes.

The selected DNA strands (genes) can be stored in gene libraries *in situ*. This technique will be particularly useful in case of vegetatively propagated crops whose *ex situ* conservation as living collections is risky and expensive. Furthermore, nucleic acid probes capable of detecting very small amounts of

the viral genome in plant material will be extremely helpful in not only detecting infected materials but would also provide a strong tool for selecting resistant genotypes.

Genetic diversity

Genetic diversity is essential for crop plant improvement. It is, therefore, necessary to maintain and further expand genetic variability to meet current and future breeding needs.

N.I. Vavilov (1926, 1951) was the first to underline the need for a really broad genetic base for crop improvement. Vavilov and his colleagues (prominent among them were Bukasov for potato collections and Kuleshov for maize collection) explored and collected cultivated plants and their wild and weedy relatives from all over the world. They studied the agro-morphological patterns of the accessions in relation to their geographical distribution, reactions to pests and diseases, and adaptation strategies. Based on these studies, Vavilov proposed the centres of diversity of crop plants and gave the concept of parallel series of variation. Both these discoveries are extremely important from the standpoint of conservation, exchange and utilization of plant genetic resources. Besides, Vavilov used the wide spectrum of genetic resources (the word genetic resources of germplasm was, however, coined much after Vavilov, in the early 1960s) in breeding programmes to develop improved varieties suitable for diverse agro-climatic conditions in the U.S.S.R.

Based on the data collected on germplasm collections, Vavilov suggested eight centres of diversity and two or three subsidiary centres of diversity. The centres of diversity (Fig. 1) were also considered as centres of origin of crop plants, and each crop has specific centre (Table 1). Variations to Vavilov's discoveries have been recorded, but the basic fact that variability in crop plants is more concentrated in certain regions than in others is a well-established and useful concept and has great implications on germplasm collection and conservation.

Table 1. World centres of diversity (centres of origin sensu Vavilov) of cultivated plants (after Vavilov, 1951)

1. The Chinese Centre

Avena nuda, naked oat (secondary centre of origin)
Glycine hispida, soybean
Phaseolus angularis, Adzuki bean
Phaseolus vulgaris, Bean (recessive form; secondary centre)
Phyllostachys spp., small bamboos
Brassica juncea, leaf mustard (secondary centre of origin)
Prunus armeniaca, Apricot
Prunus persica, Peach

Citrus sinensis, Orange
Sesamum indicum, sesame (endemic group of dwarf varieties, secondary centre)
Camellia (Thea) *sinensis*, China tea

2. The Indian Centre
Oryza sativa, rice
Eleusine coracana, African millet
Cicer arietinum, chick pea
Phaseolus aconitifolius, moth bean
Phaseolus calcaratus, rice bean
Dolichos biflorus, horse gram
Vigna sinensis, asparagus bean
Solanum melongena, eggplant
Raphanus caudatus, rat's tail radish
Colocasia antiquorum, taro yam
Cucumis sativus, cucumber
Gossypium arboreum, tree cotton, 2x
Corchorus olitorius, jute
Piper nigrum, pepper
Indigofera tinctoria, indigo

2a. The Indo-Malayan Cbntre
Dioscorea spp., Yam
Citrus maxima, Pomelo
Musa spp., Banana
Cocos nucifera, Coconut

3. The Central Asiatic Centre
Triticum aestivum, bread wheat
Triticum compactum, club wheat
Triticum sphaerococcum, shot wheat
Secale cereale, rye (secondary centre)
Pisum sativum, pea
Lens esculenta, lentil
Cicer arietinum, chick pea
Sesamum indicum, sesame (one of the centres of origin)
Linum usitatissimum, flax (one of the centres of origin)
Carthamus tinctorius, safflower (one of the centres of origin)
Daucus carota, carrot (basic centre of Asiatic varieties)
Raphanus sativus, radish (one of the centres of origin)
Pyrus communis, pear
Pyrus malus, apple
Juglans regia, walnut

4. The Near Eastern Centre
Triticum monococcum, Einkorn wheat
Triticum durum, Durum wheat
Triticum turgidum, Poulard wheat
Triticum aestivum, bread wheat (endemic awnless group. One of the centres of origin)
Hordeum vulgare, endemic group of cultivated tworowed barleys

Secale cereale, rye
Avena byzantina, red oat
Cicer arietinum, chick pea (secondary centre)
Lens esculenta, lentil (a large endemic group of varieties)
Pisum sativum, pea (a large endemic group. Secondary centre)
Medicago sativa, blue alfalfa
Sesamum indicum, sesame (a separate geographic group)
Linum usitatissimum, flax (many endemic varieties)
Cucumis melo, melon
Amygdalus communis, almond
Ficus carica, fig
Punica granatum, pomegranate
Vitis vinifera, grape
Prunus armeniaca, apricot (one of centres of origin)
*Pistacia vera,*pistachio (one of the centres)

5. The Mediterranean Centre
Triticum durum, Durum wheat
Avena strigosa, hulled oats
Vicia aba, broad bean
Brassica oleracea, cabbage
Olea europaea, olive
Lactuca sativa, lettuce

6. The Abyssinian Centre
Triticum durum, Durum wheat (an amazing wealth of forms)
Triticum turgidum. Poulard wheat (an exceptional wealth of forms)
Triticum dicoccum, Emmer wheat
Hordeum vulgare, barley (an exceptional diversity of forms)
Cicer arietinum, chick pea (A centre)
Lens esculenta, lentil (a centre)
Eragrostis abyssinica, teff
Eleusine coracana, African millet
Pisum sativum, pea (one of the centres)
Linum usitatissimum, flax (a centre)
Sesamum indicum, sesame (basic centre)
Ricinus communis, castor bean (a centre)
Coffee arabica, coffee

7. The South Mexican and Central American Centre
Zea mays, corn
Phaseolus vulgaris, common bean
Capsicum annum, pepper
Gossypium hirsutum, upland cotton
Agave sisalana, sisal hemp
Cucurbita spp., squash, pumpkin, gourd

8. South American (Peruvian-Ecuadorean-Bolivian) Centre
Ipomoea batatas, sweet potato
Solanum tuberosum, potato

Phaseolus lunatus, lima bean
Lycopersicum esculentum, tomato
Gossypium barbadense, sea island cotton (4x)
Carica papaya, papaya
Nicotiana tabacum, tobacco

8a. The Chiloe Centre
Solanum tuberosum potato

8b. Brazilian-Paraguayan Centre
Manihot utilissima, manioc
Arachis hypogaea, peanut
Theobroma cacao, cocoa (secondary centre)
Hevea brasiliensis, rubber tree
Ananas comosa, pineapple
Passiflora edulis, purple granadilla

It may be noted from Fig. 1 and Table 1 that the centres of diversity were confined to the tropics and sub-tropics and lie between 20° and 45° latitude north and south of the equator. It may further be noted that there are some notable gaps; Africa (except Ethiopia), North America and Australia are not represented in the centres designated by Vavilov. Now it is known that Australia has unique diversity for soybean and cotton; North America for *Helianthus annuus* and *Helianthus tuberosus*, and West Africa for *Oryza glaberrima* and *Dioscorea* spp.

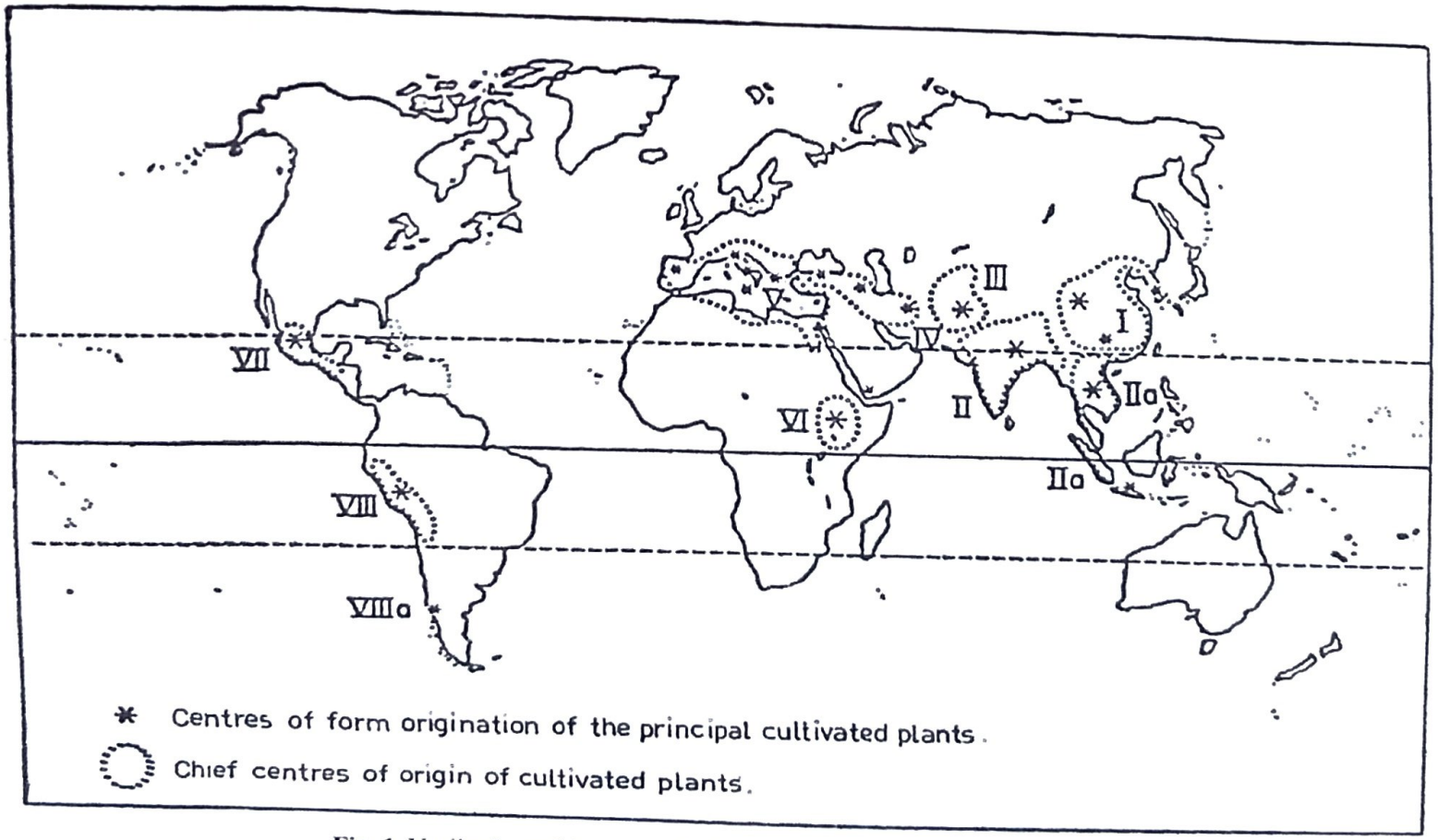


Fig. 1. Vavilov's world centres of origin of cultivated plants (Vavilov, 1951)

The pattern of genetic diversity seen in crop plants depends on actions and interactions of following factors:

- Mode of reproduction
- Pattern of distribution
- Environmental heterogeneity
- Recombination systems
- Mutation
- Migration
- Selection
- Phenotypic plasticity
- Population size

Genetic Erosion and Genetic Vulnerability

H.V. Harlan was the first to express concern that there was a danger of losing genetic diversity (Harlan and Martini, 1936). In the post World War II era, loss of land races and other germplasm became alarming when the high yielding varieties of rice and wheat were introduced into areas of traditional agriculture for increased production. In the centres of diversities of these crops, the primitive cultivars and their wild and weedy relatives were getting replaced at a very high rate. This unprecedented loss was christened as genetic erosion. The quest for development has not only accelerated plant genetic erosion but the adoption of modern agricultural practices has also rendered the major crops genetically vulnerable to biotic and abiotic stresses. Famines and large-scale devastations of agricultural production have occurred due to genetic uniformity. During the past 50 years, following are some of the major destructions: (i) the Bengal famine of India in 1943 associated with the brown spot disease of rice *(Cochliobolus miyabeanus),* (ii) the complete elimination in the mid-1940s of all oats derived from the variety: Victoria in the U.S.A., due to the Victoria blight disease caused by *Cochliobolus victoriae,* (iii) the epidemics of Southern corn leaf blight in the U.S.A. caused by *Cochliobolus heterostrophus* during 1970–71 on maize hybrids that have the T-cytoplasm conferring male sterility, and (iv) the fast emergence of biotype 2 of rice brown planthopper (*Nilaparvata lugens*)from biotype 1 in mid-1970s and newer biotypes in the mid-1980s in Southeast and South Asia when large areas were planted to a few semi-dwarf rice. Even the multiple resistance of IR 36 broke down under mono-variety multiple cropping in Indonesia during 1986, let alone the resistance of varieties possessing narrow genetic bases.

Genetics Resource Activities

While development processes will continue to intensify and there will be concommitant losses of genetic resources, to conserve genetic diversity essential interrelated genetic resource activities must be undertaken, as, for example:

i) Exploration and collection;
ii) Conservation;
iii) Characterization and evaluation;
iv) Documentation; and
v) Utilization.

Interaction between different activities is shown in Fig. 2.

Exploration and collection

Systematic exploration and collection is a highly scientific process. A genetic resource sample should contain the fullest possible representation of genetic variability of the target population. In other words, the sample should contain representative allelic frequencies and adaptive complexes. In order to achieve this, an understanding of genetic architecture, adaptation strategy, pattern of ecotypic differentiation, and eco-geographical distribution will be necessary. Depending on the eco-genetic attributes of the population, the sampling strategy (including sample size and collection procedures) will vary from species to species and location to location.

Before launching a collection mission, it is necessary to: (i) survey the past efforts and current status of conservation of germplasm of the crop(s) under consideration for assessing major gaps in the existing collections on the bases of crop geography, bio-systematics, and crop ecology; (ii) set up collection priorities with regard to type of germplasm to be collected and the geographic areas to be covered; (iii) formulate sampling strategy; (iv) constitute an effective collection team comprising trained scientists; and (v) arrange adequate funding and other logistic support including full involvement of the host country if the collection is to be made abroad.

The following criteria are used for fixing collection priorities.

i) Degree of threat of genetic erosion;
ii) Adequateness of existing collections from the point of view of representation of the known magnitude and pattern of variation of a given crop and its allies;
iii) Needs of plant breeders and other researchers for specific types of germplasm;

iv) Economic importance of the crop under consideration; and

v) Richness of genetic and environmental settings and their accessibility to the collector.

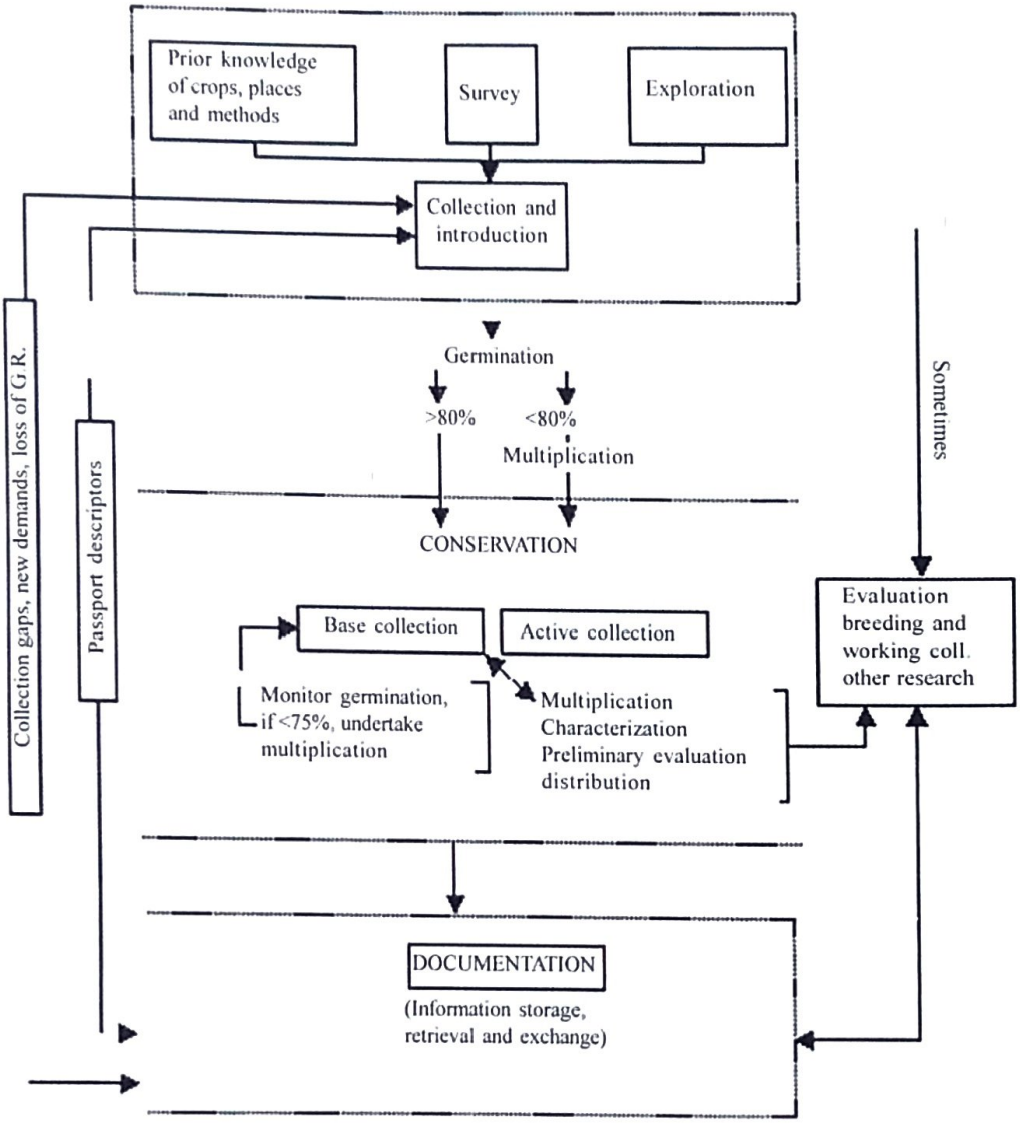


Fig. 2. Linkages among plant genetic resource activities.

Sampling strategy

The sampling strategy will differ according to the purpose of collection. Generally speaking, collection should be seen as a "rescuing" as well as a "now or never" operation (Hawkes, 1981). In this context, a random or non-selective sampling strategy designed to capture the greatest range of genetic diversity in the minimum number and size of samples should be adopted.

Selective or non-random and mission-oriented samplings have been advocated sometimes. In this approach, only certain visibly discernible morphotypes are collected. With selective sampling, variation in disease resistance alleles or genes for adaptation to stress conditions are not sampled adequately. A mixture of selective and non-selective samples has also been suggested (Bennett, 1970) with the assumption that while selective samples would provide the sought-for genes, the random samples would ensure capturing of alleles for biotic and abiotic stresses.

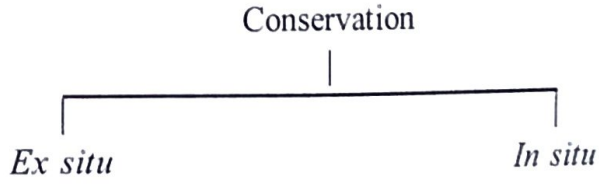
As regards sample size, Marshall and Brown (1975) suggested that an optimum strategy should enable the collector to capture, with 95 per cent certainly, all the alleles occurring in the population at a frequency greater than 5 per cent. To achieve this, 50-100 individuals should be collected per site with about 50 seeds taken from each plant. Furthermore, the collecting site should be scattered throughout the range of species, including the peripheral areas of distribution of the species.

Sampling of vegetatively propagated crops is more difficult than that of grain crops. Random sampling of vegetatively propagated populations in the field may not be effective because many populations may originate from a single or a few clones. Therefore, selective sampling to obtain distinct morphotypes from different collection sites would be more appropriate. Wherever possible, the collection of vegetative propagules should be supplemented with collection of seeds.

Conservation

Conservation could be for varying periods: short, medium or long. The nature of the material to be conserved and the objectives of conservation would decide the strategies of conservation. The length of life cycle, the mode of reproduction, the number and size of individual plants to be conserved and the evolutionary status (wild or domesticated) of the material determine the mode of conservation.

Conservation can be classified into two major categories, *in situ* and *ex situ*. The latter can further be divided into five sub-categories as follows:



i) Long-term seed storage
ii) Mass reservoirs of composites
iii) Field conservation
iv) Cold storage freeze preservation of vegetative parts
v) *In vitro* conservation

In Situ Conservation

In situ conservation is an approach in which the species are conserved in their natural state through the establishment of nature or biosphere reserves, national parks, or by special legislation to protect endangered or threatened species. In this system, wild species and the associated natural ecosystems are preserved together to maintain the genetic integrity of the population and to allow natural evolution. The *in situ* conservation approach is not suitable for cultivated forms as there are no natural eco-systems to support them, although some workers had suggested that small areas could be preserved in which land races could be cultivated according to traditional methods to allow evolutionary processes to continue. But, from the socio-economic point of view, this approach would generally not be acceptable. With ever-shrinking per capita availability of land, especially in South and Southeast Asia (where arable land is most scarce), it would be difficult to set aside large areas for cultivation of low-yielding land races.

Ex Situ Conservation

Seed genebanks

The most practical and the cheapest method of conservation of genetic resources of species producing orthodox seeds is through long-term cold storage of seeds. Orthodox seeds are those which tolerate a decrease in moisture content under low temperature. Such seed banks are also called gene banks. In case of those species which produce recalcitrant seeds, the seeds cannot be stored in gene banks, because recalcitrant seeds do not stand drying below a relatively high moisture content without a serious loss of viability (Roberts, 1973), such as, in the case of cocoa, mango, durian and several other tropical fruits. Among species bearing orthodox seeds, there is genetic variation in storability; other things being equal, some species can be stored for longer periods than others. For instance, in leguminosae family, seeds of *Cassia, Trifolium,* and *Lupinus* species could be cold-stored for over 100 years, whereas the seeds of groundnut have rather short cold-storageability.

Depending on the duration of conservation, there are three types of conservation, namely, base collection, active collection, and working collection, as briefly described here:

Base collections: These comprise long-term stored materials which are disturbed only for purposes of regeneration. The materials conserved in base collections provide safety of genetic resources over generations. Base collections contain seeds with 5 ± 1 per cent moisture content placed in sealed containers and stored at –18°C to – 20°C.

Active collections: These comprise medium-term (10 to 15 years) storages and are used for the regeneration, multiplication, distribution, characterization and documentation. Seeds in active collections are stored at temperature around 0°C and moisture content of around 8 per cent.

Working collections: These comprise the genetic resources which are regularly used by the plant breeder, pathologist, entomologist, etc., who have no responsibility for maintaining them. However, even such materials also need not be grown every year and are required to be stored for three to five years. For such a storage, seed moisture content of about 8 to 10 per cent and temperature of 5°C to 10°C is required.

During storage in a gene bank, it is necessary to control temperature and humidity to maximize the longevity of the seeds. Harrington (1963) indicated that seed longevity is doubled for each 5°C fall in temperature or for each 2 per cent drop in moisture content. This, he called, a "rule of thumb". This rule applies between 1°C and 50°C for temperature and between 5 and 14 per cent for seed moisture content. There is little interaction between the temperature regime and moisture content. In other words, the two rules based on temperature and moisture content apply independently.

Mass reservoirs

Simmonds (1962) suggested the use of mass reservoirs or composite crosses of large number of diverse parents as a means of long-term conservation. Such reservoirs are subjected only to natural selection and thus remain under an evolutionary stage. It was suggested that, based on ecological, agromorphological and genetical similarities, different accessions could be bulked to constitute a mass population. Mass reservoirs, originating from intermating a set of parents, constitute a created genepool.

Field genebanks

Many crop species, especially the perennial tree crops, produce recalcitrant seeds, and some of them even cannot be multiplied vegetatively. Hence, of

necessity, such species are to be conserved as living field collections. Large scale *ex situ* conservation plots of vegetatively-propagated perennials have been or are in the process of being established in several countries. Malaysia has already put 500 hectares under oil palm germplasm. Indonesia has earmarked almost 1,000 hectares for conservation of coconut and other perennial crops conservation (Singh 1982) Philippines is maintaining *ex situ* field collections of Southeast Asian germplasm of bananas. India has established a global collection of coconut germplasm in the Andaman and Nicobar Islands. Maintenance of such collections is rather costly and open to attack by diseases and pests; thus it calls for careful management. On the other hand, it has the advantage of being continuously evaluated, and desired accessions can immediately be utilized directly or in breeding programmes. From the point of view of genetic conservation, field genebanks are, in most cases, totally unrepresentative of the range of genetic variability within the respective crop genepool and most of them do not constitute more than a fraction of the variability which should be conserved for the future.

Cold storage and freeze preservation of vegetative parts

In the case of vegetatively-propagated crops, vegetative propagules such as rhizomes, corns, tubers, and cuttings are usually short-lived and deteriorate fast after collection, unless stored under appropriate conditions. Low temperature, (but not lower than 14°C) coupled with high humidity stores yam and sweet potato propagules rather satisfactorily for several months. Potato tubers can be stored adequately for five to seven months at 4-5°C and 90 per cent RH. But, these techniques do not free the curator from the job of raising the stocks every year or once in two years, which is not only costly but is also fraught with dangers of disease and pest attacks and many other hazards. Methods of medium and long-term storage such as cryo-preservation and *in vitro* conservation can be utilized for vegetatively propagated species.

In vitro conservation

In vitro conservation is an important adjunct to usual conservation methods especially for those species which cannot be conserved through orthodox seeds or whose seed production is very poor. Further, the tissue cultured materials are not exposed to pests and pathogens which usually appear on field collections. The materials can be stored at ultra low temperatures of liquid nitrogen (–196°C), thus giving high genetic stability and very long regeneration cycles. Finally, *in vitro* culture technique avoids the need of going through sexual cycle of about 10 to 20 years in several tree species. However, this technique is applicable only to those species whose tissue culture and regeneration techniques are fully standardized. Some important vegetatively propagated species *viz.*

mango, durian, and guava are yet not amenable to *in vitro* culture and would have to be maintained in field genebanks.

In vitro genebanks may also be grouped into two categories: (i) the *in vitro* base genebanks where cultures are maintained under conditions of cryopreservation; such genebanks are as yet non-existent, and (ii) the *in vitro* active genebanks where cultures are maintained under slow growth, such as the existing collections of cassava at CIAT and IITA, potato at CIP and sweet potato at CIP, AVRDC and IITA. As depicted in Fig. 3, these genebanks are expected to be linked with breeder's working collections and field genebanks.

Conservation should ensure genetic fidelity of the material stored. The risk of genetic instability of *in vitro* stored material can range from minimal to considerable depending upon the culture systems used. Figure 4 shows the different *in vitro* culture systems available for use in genetic conservation. Other things being similar, tissue culture systems derived from shoot, internodes or other aerial parts show greater instability as compared to those derived from root culture (Chaturvedi, 1987).

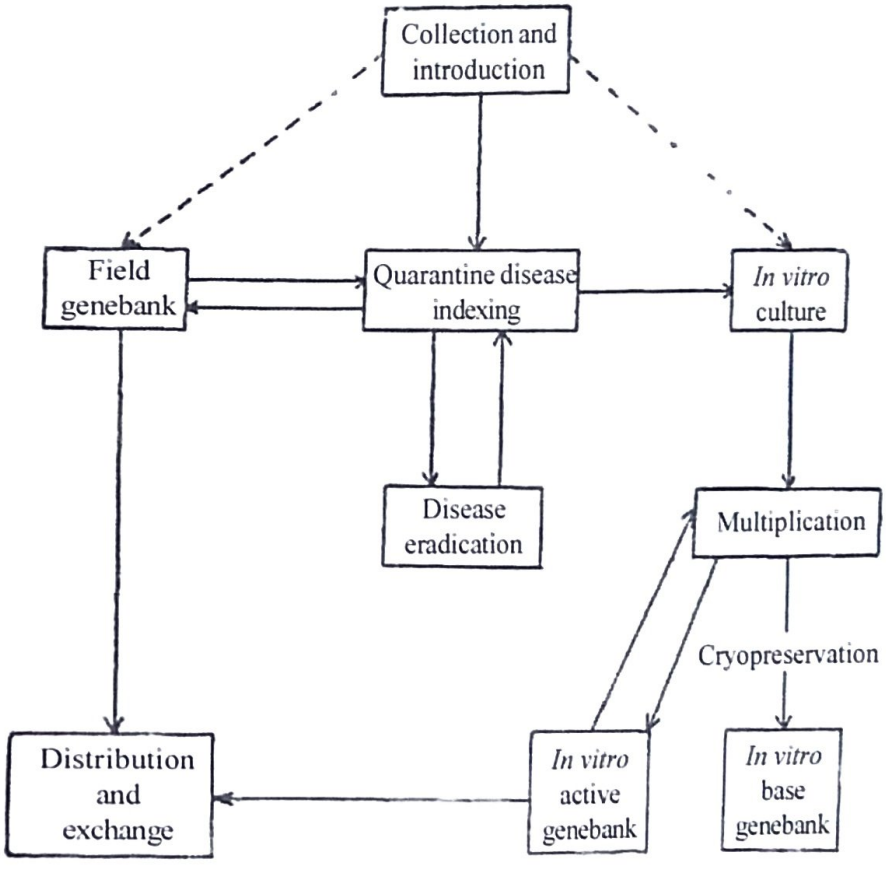


Fig. 3. Linkages among collection, conservation (base collections, active collections) and distribution of germplasm using *in vitro* techniques.

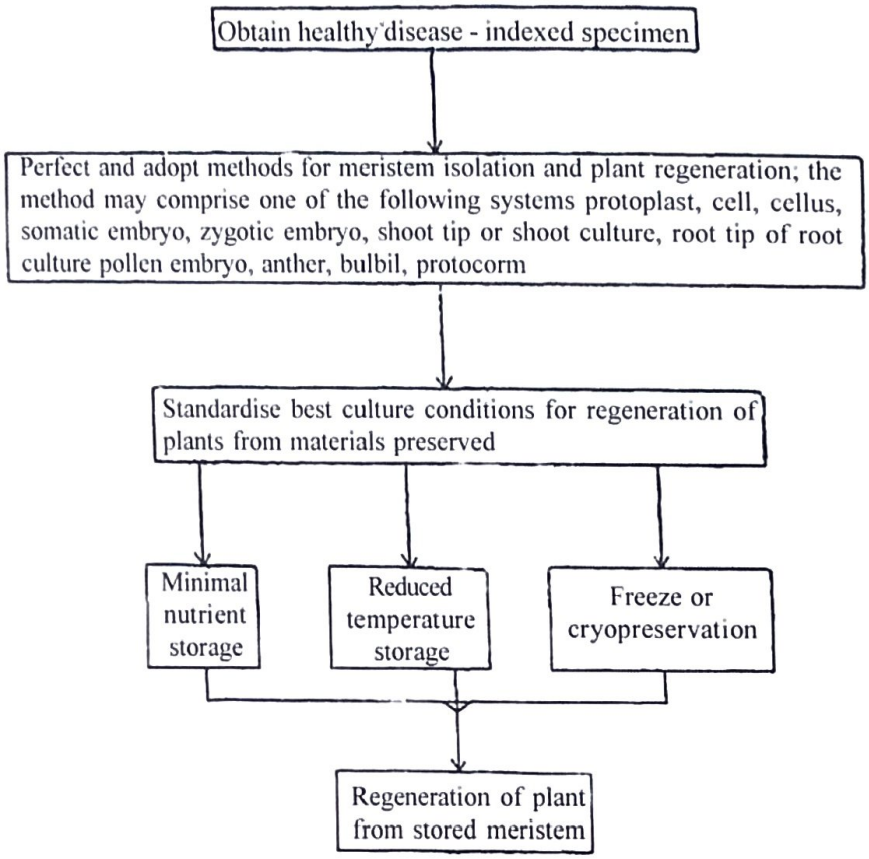

Obtain healthy disease - indexed specimen
Perfect and adopt methods for meristem isolation and plant regeneration; the method may comprise one of the following systems protoplast, cell, cellus, somatic embryo, zygotic embryo, shoot tip or shoot culture, root tip of root culture pollen embryo, anther, bulbil, protocorm
Standardise best culture conditions for regeneration of plants from materials preserved
Minimal nutrient storage
Reduced temperature storage
Freeze or cryopreservation
Regeneration of plant from stored meristem

Fig. 4. Flow chart of the mode of *in vitro* conservation.

Maintenance of genetic resources

Over a number of decades many collections have been lost thus negating the scientific and financial outlay involved in their formation. Even large centres with modern facilities and adequate expertise have reported up to 50 per cent loss of samples from their original collections. The genetic fidelity of a sizable proportion of the older collections is often questioned. Further, most of the older collections contain an appreciable percentage of redundant duplicates.

Such problems can be the result of the incorrect choice of sample size for multiplication, or of multiplication or regeneration in the wrong environments, lack of attention to the breeding system of the crop during multiplication and regeneration and numerous other factors. In fact, the methods of maintenance of material have not received the attention they deserve.

If germplasm is maintained correctly, the genetic constitution of the population at the time of its collection should be preserved. It is presumed that the original population was efficiently sampled and that the sample contains representative allelic frequencies and adaptive complexes. Nevertheless, the maintenance of genetic integrity depends on how closely population genetics theory, biological,

ecological and agricultural properties of the collections, the intended uses and the practicalities of multiplication are taken into account (Singh and Williams, 1984).

Characterization and evaluation

Characterization means recording of state of those characters which are highly heritable, can be seen by the naked eye, and are expressed in all environments. Characterization is usually done by curators during multiplication of accessions.

Preliminary evaluation is made on a limited number of additional traits though desirable by a consensus of users of a particular crop. These traits can be assessed visually, but may not be expressed in all environments, for example reaction to diseases and other stresses. Preliminary evaluation is often done by the curator by growing non-replicated observation rows.

Full evaluation is done by breeders in association with other allied disciplines and includes both quantitative and qualitative traits and those characters which are of interest to the breeder. This is usually based on multilocation multi-year replicated trials. For full evaluation, a multi-disciplinary team and an inter-disciplinary approach is needed, as is in vogue at International Rice Genebank at IRRI and in some of the national plant breeding programmes.

Documentation

Documentation is an essential component of all genetic resource activities and provides the linkage among curators, plant breeders and other researchers. Systematic documentation of conserved materials and exchange of information stimulates germplasm exchange, evaluation as well as utilization. Hence an efficient system of data storage, retrieval and dissemination should be established.

Documentation could be manual or machine-based. For larger collections, such as the IRRI collection of rice where more than 70,000 accessions are held and for each accession about 45 descriptors (traits) are to be recorded, the volume and complexity of the data is very high and could not be handled manually. For such cases, suitable machine-based data management systems should be available.

Utilization

Genetic resources are to be utilized for developing newer varieties to meet the ever-changing needs of mankind. As mentioned earlier, conservation, characterization, evaluation and documentation are all geared to facilitate effective and efficient use of genetic resources. Generally, the following factors influence extent of utilization of germplasm by plant breeders:

1) food and agro-industrial development demands,
2) breeding objectives and strategy,
3) availability of the material and information thereon,
4) manpower, infrastructure, personality and background of the breeder, and
5) level of research support, including inter-disciplinary research.

To what extent various germplasm collections have been utilized varies from crop to crop, station to station and country to country. Utilization of germplasm essentially means plant breeding. In case of pasture, forest, and fruit species, the cultivated or domesticated types are fairly close to their counterparts in wild conditions, and introductions of such species are used directly for identifying new cultivars. In case of field crops, on the other hand, gene introductions are mainly used in recombination breeding, with exception of occasional introduction of improved cultivars from analogous agro-climatic conditions, *viz.*, international distribution of modern varieties of rice and wheat in the 1960s.

New frontiers in genetic resource activities

In vitro culture, including cryopreservation, holds several new promises for germplasm activities. *In vitro* techniques for virus indexing and safe transfer of germplasm and planting materials of cassava (Kartha, 1981), potato (Huaman, 1986) and sweet potato (Ng 1987) have been extremely effective in the sharing of genetic resources of these crops. For other root and tuber crops, the system is yet to be made foolproof and further research and development work is called for. For fruit trees and forest species, the technique is standardized only for a few species and a lot more work is needed. Somatic embryogenesis techniques have been streamlined for several crop plants. Further, individual embryos can be encapsulated in suitable materials to form artificial seeds which can withstand the rigours of handling during transportation (Bhojwani and Dantu, 1987).

In vitro techniques are important not only for conservation of genetic resources, but can also be used for the creation of new and novel variability. Somaclonal variations, documented in several cases, show that new variability within the co-adapted gene complexes of many of the modern cultivars can be created (Scowcroft *et al.*, 1984). In this way, already successful cultivars can be improved progressively.

Rapid developments in biotechnology, particularly recombinant DNA technology, in the recent years have great implications on conservation and utilization of the genome (Peacock, 1984; Chopra, 1985; Ranjekar (1987). Using the recombinant DNA methodology, nucleic acid probes can detect very small amounts of the

viral genome in plant material and provide quarantine safety. The same probes will also provide tools for screening breeding materials for disease resistance.

Genomic as well as cDNA libraries are now distinct possibilities. It is hoped that in the not-too-distant future it should be possible to isolate and transfer specific genes to meet specific needs of crop production, without causing disruption of the existing selected and adapted genomes. The DNA technology is expected to greatly extend the possible sources of genes for transfer into any given crop species far beyond the barriers set by sexual hybridization. Clearly, biotechnology and *in vitro* techniques would be one of the most potent aids to conventional methods of conversation and utilization of crop genetic resources.

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14

Innovative Approaches for Crop Improvement

V.L. Chopra and R.P. Sharma

In conventional breeding for genetic upgradation of yield and other desirable attributes of crop plants, favourable assemblages of genes from two or more parents are made to develop a commercial variety. The important steps of this exercise are: locating the right kind of parents that possess the desired characteristics; making crosses between or among them; handling the segregating generations in an appropriate manner so as to identify the required recombinants; stabilizing the recombinants and finally evaluating their performance in a series of trials. This approach has been very successful in the sense that it has provided increments in productivity continuously. In an analysis done by the American Society of Agronomy on the genetic contributions to yield gains of five major crop plants, it was concluded that sorghum yields increased due to genetic improvements at the rate of 1.2 per cent per year between 1950 and 1980. For corn, the corresponding yield increase was 90 kg/ha/year between 1930 and 1980, which is equivalent to a rate of increase of 1 per cent per year. It was also estimated that 70 per cent of the total gains in corn yields are attributable to genetic improvement and 30 per cent of the yield gains are due to non-seed inputs like fertilizers, pesticides and cultural practices.

In India also, the improvement of productivity of the major food, fibre and several cash crops has been very impressive. The success is due to intensive research in plant breeding resulting in the evolution of varieties with very high genetic yield potentials and the concurrent support from other disciplines that has provided the package of practices which help in the realization of the created potential. In all crop plants, where the breeding efforts have been intensive, the phenomenon of plateauing of yields has become apparent. Wheat provides, perhaps, the best example of the situation. Since the 1970s, when the gains from genetic restructuring of the plant type had been established, major yield

increases have not occurred. Since the demands of the future will be undoubtedly much larger, and additional cultivable land will not be available, ways will have to be found for a sizable increase in productivity per unit area in the immediate future. This requirement calls for the development of strategies and procedures that will make dramatic yield gains possible. For meeting the challenge, a continuous search is being made for innovative methods which will give yield increments of a much higher order than those provided by the conventional approach. In this chapter, an attempt has been made to introduce some such promising approaches.

1. Exploitation of heterosis in self-pollinated crops

Traditionally, heterosis has been exploited in cross-pollinated crops because of the ease with which hybrid seed can be produced in abundance at reasonable cost. In several major self-pollinated crops, heterosis has been experimentally demonstrated though, till recently, its exploitation was confined only to some vegetables. More recently, exploitation of heterosis has been extended to self-pollinated grain crops also. A major factor contributing to this success is the discovery of exploitable male sterility which helps in the production of F_1 hybrid seed without the necessity of manual emasculation and pollination.

Usable male sterility, in the plant breeding sense, is the selective damage to the functioning of the male gamete without an adverse effect on female fertility. Three major types of heritable male sterility are found in crop plants.

1) Genetic male sterility

Genetic male sterility is controlled by nuclear genes. Most genetic male sterilities are monogenic recessive, although there are some reports of control by two or more genes. Recessive genetic male sterility has been reported in tomato, sorghum, barley, rice, wheat, pigeon pea, brassica, sunflower, watermelon and lettuce. Male sterility controlled by single dominant gene has been reported in cotton and wheat. Genetic male sterility controlled by recessive genes can be maintained only in the heterozygous condition. Its commercial exploitation, for hybrid seed production, is not easy because of two reasons: (i) only a fraction (1/4 of the F_2 in the case of a single recessive gene) is pheno-typically male sterile and can be identified only at flowering, and (ii) the sterile plants are dispersed in the segregating population and the necessity of handling them individually does not allow economic seed production. For these reasons it has not been possible to employ genetic male sterility for producing seeds of commercial heterotic hybrids, even though schemes for utilizing genetic male sterility of the XYZ system in wheat have been proposed (Driscoll, 1972). The X component of the scheme represents two doses of rye chromosome carrying the fertility gene corresponding to Ms for sterility and a genetic marker, hairy

peduncle. Y has only one dose of alien rye chromosome so that it produces two types of male gametes (with alien, which is non-functional; without alien, which is functional). Similarly two kinds of female gametes are produced: (i) those with the alien chromosome, which on fertilization gives rise to a 'Y' plant, and (ii) without alien, which is non-hairy and is sterile. This genotype without a dose of alien chromosome is the Z component. In this scheme, X & Y components are needed only for the production of female parent of the hybrid while Z, which has normal chromosomal constitution, bears the hybrid seeds (Fig. 1).

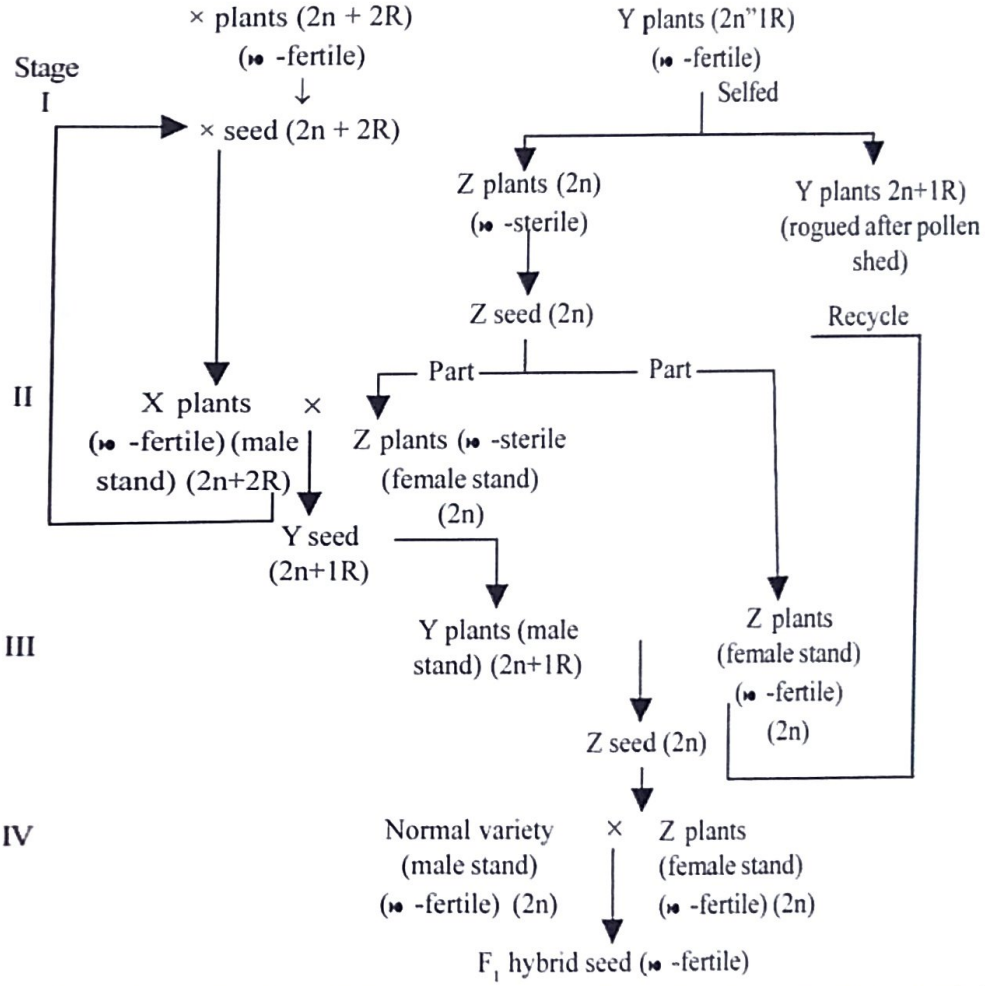

Fig. 1. Steps in production of hybrid wheat using XYZ genetic male sterility (Driscoll, 1972).

2) Cytoplasmic male sterility

The control of male sterility in this case resides in the plasma genes of cytoplasmic organalles. Since in plants pollen does not contribute to the cytoplasmic content of the offspring, the sterility controlling genes are contributed only by the female parent. Therefore, even when a pollinator is available, all progeny of the cytoplasmically controlled male sterile is sterile in the next generation. Cytoplasmic male sterility has been reported in *Petunia, Beta vulgaris, Nicotiana* and *Capsicum*. The maintenance of cytoplasmic male

sterility is easy. Sterility is maintained by pollinating the male sterile seed parent with viable pollen. Cytoplasmic male sterility can also easily be transferred into any genotype by crossing a male sterile with the genotype to which the sterility is to be transferred followed by a backcross procedure using the male genotype as a recurrent parent. Cytoplasmic male sterility can be exploited commercially when vegetative parts, rather than seed, is of commercial value. It has also been utilized for hybrid ornamentals where the total requirements of seed are small and the pricing structure is very favourable.

3) Cytoplasmic-genetic male sterility

In the cytoplasmic-genetic male sterility, the interaction between the plasma genes and the nuclear genes determines the pollen fertility of the plant. Genes in the cytoplasm can cause male sterility in certain nuclear backgrounds while in others they are rendered ineffective so that a plant is male fertile in spite of them. The pollen fertility behaviour of the combination of cytoplasmic and nuclear genes, and the terminology used to describe them, is given in Table 1.

Table 1. Possible cytoplasmic-genetic constitutions of plants and their pollen fertility behaviour and accepted designation.

Cytoplasm	Nucleus	Pollen fertility behaviour	Designation of line	Symbol
S	rf rf	sterile	CMS	A
N	rf rf	fertile	Maintainer	B
S/N	Rf rf	fertile	Restorer	R
S	Rf rf	fertile	Hybrid	A × R

Source: Genetic Manipulation for Crop Improvement, V.L. Chopra (ed.).

Cytoplasmic-genetic male sterility is of very wide occurrence and has been reported in 147 species of 47 genera of 20 families of angiosperms. Crop plants in which cytoplasmic-genetic male sterility is known include maize, sorghum, pearl millet, wheat, rice, barley, cotton, tobacco, sunflower, brassicas and some vegetables (tomato, carrot, radish, pepper, onion).

In self-pollinated crops, like in their cross-pollinated counterparts, cytoplasmic-genetic male sterility has been utilized in two ways: (i) production of F_1 hybrid seeds, and (ii) recurrent selection procedures. While the first exploits combining ability, the second aims at concentrating frequencies of favourable genes so that the population gives a better mean performance but still retaining genetic variability. Because of this variability, the material so generated can be further improved through additional cycles of selection. In India, cytoplasmic-genetic

male sterility has been exploited at the commercial scale in pearl millet and sorghum. Efforts are also afoot for exploitation of heterosis in rice following the success of similar effort in China.

2. Androgenic haploids and their use in breeding

An important step in breeding self-pollinated crop plants is the attainment of homozygosity following the isolation of recombinants. Conventionally, this has been done by successive selfings after a recombinant has been detected. Haploids originating from the male gametes offer two distinct advantages: (i) Colchicine-induced doubling of the haploids will generate homozygosity in one step, and (ii) when practised on a large scale using anthers of a hybrid, it combines the advantages of recombination, segregation and instantaneous fixation. For the production of androgenic haploids, cultures are established either from anthers or from isolated microspores grains When anthers are inoculated on appropriate media, they dehisce and somatic embryos appear. These embryos are at the cotyledonary stage, resemble the zygotic embryos morphologically and germinate to give rise to haploid seedlings. In other species, the pollen from the anthers can be shed in liquid medium and embryos developed from the pollen after removal of the anthers. Pollen grains isolated after homogenization and centrifugation have also been demonstrated to develop, in some cases, into pollen embryos. In most cases, however, the efficiency of haploid production in such cases is low.

Haploids are a very good material for the induction and isolation of mutants which can be used for basic work or for plant breeding. Mutants have been isolated for such characteristics as resistance to methionine-sulphoximine and the linked resistance to wild fire disease caused by *Pseudomonas* in tobacco, resistance to NaCl in tobacco and resistance to phytopathotoxins, herbicides, environmental stresses and amino acid analogues.

It has been argued that introduction of the androgenic haploidy followed by doubling can reduce the time required for developing a variety from the normal eight to 10 years to five to six years. *In vitro* produced haploids have been successfully exploited for developing crop varieties. In Japan, the tobacco variety F 211 which is resistant to bacterial wilt is a derivative of anther culture haploids. In China, 81 varieties and strains of rice and 20 varieties of wheat have been derived from androgenic haploids and are very successful commercial varieties. The rice variety "Hua Yu No. 1" possesses high yield, resistance to bacterial blight and wide adaptability. Another variety of rice, "Xin Xiu", is cultivated on more than 100,000 hectares. Wheat varieties developed through anther culture technique include "Huapei 1", 'Yunhua 1' 'Yunhua 2' and 'Jinghua 1. The desirable characteristics of these varieties are early maturity, rust resistance, high thousand grain weight and cold resistance.

3. Distant hybridization

In crop plants, several characteristics of economic value, such as resistance to diseases and environmental stress, are present in wild and weedy relatives. The transfer of these characteristics through conventional breeding approach is difficult because of compatibility barriers imposed by failure of pollination or embryo development, variations in chromosome number and lack of chromosome pairing and consequent sterility etc. To overcome these barriers two broad approaches have been employed: (1) Embryo rescue, and (2) Somatic hybridization.

Embryo rescue

Embryos, developed in distant crosses, suffer from post-fertilization developmental blocks. This prevents the formation of normal hybrid seeds. The developing embryos are heterotrophic and the nutritional supplies are provided by the endosperm. In intergeneric crosses, the endosperm disintegrates and results in the collapse of embryo. The hybrid embryo can be rescued from collapse by allowing it to grow on an artificial medium. This can be practiced by following either of the following three methods: (a) Embryo culture; (b) Embryo implantation; and (iii) Ovule culture.

a) *Embryo culture* technique in hybrid embryo rescue approach, that leads to development of normal hybrid seedlings, has been practiced in a number of interspecific crosses. In legumes, crosses involving *Phaseolus vulgaris* × *Phaseolus vitensis, Arachis hypogaea* × *A. montiola* or *A. glebrata;* in cereals, *Triticum aestivum* × *Aegilops speltoides, Triticum durum* × *Secale cereale, Hordeum vulgare* × *Secale cereale, Aegilops squarrosa* × *Triticum boeoticum*,embryos have been successfully rescued and seedlings established from them.

b) *Embryo implantation:* The success of embryo culture technique depends on stringent nutritional requirements which generally differ from species to species and therefore require standardization in each case. This limitation can be overcome by transplanting hybrid embryo, before their collapse, into a normal endosperm. This technique has been successfully employed in crosses involving *Hordeum* × *Triticale, Hordeum* × *Agropyron* and *Hordeum* × *Secale.*

c) *Ovule culture:* To overcome the difficulties of arriving at the right kind of complex nutrient medium for culturing hybrid embryos and for avoiding the difficulty of dissecting out uninjured embryos at the right stage for the purposes of transplantation, culture of the ovules has been suggested as an alternate method. Nutrient requirements for ovule growth and embryo differentiation are surprisingly simple and include amino acids and hormones. Cotton is a

good example of a crop plant in which ovule culture has been used to rescue hybrid embryos. In cotton, *Gossypium arborium* and *G. herbasium* are diploids while *G. barbadense* and *G. hirsutum* are allotetraploids. In attempts to produce hybrids between diploid and tetraploid species, abnormalities of endosperm growth lead to embryo abortion when tetraploid is used as the maternal parent; when the diploid is used as the female parent, both embryo and endosperm show abnormal growth. Hybrid embryos from tetraploid-diploid crosses have been grown to maturity in specified culture media: Rescue of embryos of the reciprocal crosses (diploid × tetraploid) are successful only sporadically.

Another technique for the success of wide crosses is culture of ovaries instead of ovules. In crosses of *Brassica campestris* × *Brassica oleracea,* seeds with well-developed embryos have been obtained when ovaries were cultured four days after pollination in white's medium containing cacsine hydrolysate.

Somatic hybridization

As an alternative to sexual hybridization, somatic hybrids can be produced by fusing protoplasts of the two donor parents. Plant protoplasts are cells in which the cell wall has been removed by treatments with enzymes like cellulase and pectinase. The initial interest was to fuse cells to produce hybrids which are not possible by sexual crosses. It has, however, been found that it is impossible to make meaningful use of such hybrids for crop improvement work because what is required is only a few genes of interest from a donor parent rather than its complete genome. Efforts are, however, continuing to devise techniques of limited gene transfers through protoplast fusion.

More recently, emphasis of somatic hybridization has shifted to the production of cytoplasmic hybrids (cybrids) and variable combinations of nuclear and organallg genomes. The advantages of this approach are: (i) since organalle replication is not synchronous with nuclear division, the organalle assort randomly in the progeny hybrid cells. The process of sorting out is rapid if a selectable marker is available. For example, selection in the presence of streptomycin will force sorting out of streptomycin-resistant chloroplast, (ii) Mitochondria *(mt)* and chloroplast *(cp)* sort out independently. Triazine (a herbicide) resistance is chloroplastborn. Male sterility genes reside in mitochondria. When a triazine resistant protoplast is fused with a protoplast of male sterile line, and selection is made for male sterile cytoplasm, both triazine resistant and triazine sensitive lines are recovered (*i.e.* both parental type chloroplasts). Somatic hybrids, thus, generate combinations of mitochondrial and chloroplast genomes, not possible through sexual means. The variation in cytoplasmic genes is further increased by recombination between *mt* DNA in cybrids.

An interesting application of somatic hybridization has been demonstrated by Pelletier and coworkers (1983), in France, who combined two different traits into one breeding line of rape seed (*Brassica napus*). By conventional breeding, lines with *Brassica napus* nucleus and *Brassica campestris cp* and *mt* have been produced which show resistance to herbicide triazine. A *Brassica napus* line is also available that contains radish *(Raphanus sativus) cp* and *mt*. This line is male sterile because of *mt* borne gene from *Raphanus mt*. A fusion of protoplast of the two lines produced cybrids that contain a *B. napus* nucleus, *R. sativus mt* and *B. campestris cp*. The cybrid plants express both male sterility and triazine resistance. Such a breeding line, containing *cp* of one genus and *mt* of another genus, could not have been produced by conventional breeding.

4. Improving selection efficiency through modern methods

The reliability of the selection procedure is crucial to the success of a plant breeding programme. In the early years, the sole criterion of selection was breeder's judgement based on the conformity of the selected phenotype to a conceptual productive or otherwise desirable combination of plant characteristics. The visual criteria of selection are subjective by nature. Molecular probes are currently lending precision to the selection process. In triticales, for example, a major problem is the selection of genotypes in which the endosperm develops normally to produce plump grains. It has been found that the failure of grain development of triticale arises from disjunctional difficulties introduced by the terminal heterochromatin of the rye chromosomes. Radioactively labelled probes have been constructed and used to screen for Triticale genotypes lacking the terminal heterochromatin. The grain development in these selections has been good.

Labelled probes, based on cDNA, are being routinely used now for selecting recombinants resistant to viruses in potato. In breeding potatoes resistant to viruses, an important objective is to reduce the time between identification of the desirable recombinant and ascertaining its virus resistance status. In conventional breeding after a recombinant with desired productivity traits is isolated, its resistance to viruses has to wait till the clone has been multiplied sufficiently for the testing procedure to be operated. The use of molecular probes cuts short this period so that testing for virus resistance can be done within a year. The strain to be tested is grown very close to a susceptible variety to ensure that the vector transmits the virus to all plants. Sap from the plants to be tested is fixed onto nitrocellulose filter and is incubated with the radioactive probe (DNA complementary to the RNA of the virus under test). Hybridization indicates the presence of virus in the plant. Selections are made of plants that show no hybridization. This procedure is called sap-spot test and allows screening of a large number of samples in a day.

In Western countries, the bread-making quality of wheat is an important consideration. Since the bread-making test requires sufficient flour, selection for this attribute is not possible during early generations.

Combined genetic, biochemical and dough-making analyses have established a correlation between glutanine subunits in wheat grains and the viscoelasticity of the dough, and hence its bread-making quality. The glutanine subunit test can be successfully run even with the half portion of a grain not containing the embryo. This biochemical parameter, therefore, provides an indirect selection criterion in breeding for the bread-making quality of wheat that can be operated during the early segregating generations.

5. Somaclonal variation

An aspect of plant tissue culture technology which at present is being strongly recommended for crop improvement is somaclonal variation. Somaclonal variation refers to heritable changes which accumulate in the callus (differentiated growth) from a somatic explant and expresses in the progeny of *in vitro* regenerants obtained from the callus. Even though relative data from deliberately designed experiments is not yet available, indications of possible advantages of somaclonals vis-a-vis induced mutagenesis are: (i) the frequency of variation seems to be far greater than the yield of induced mutations; (ii) the changes are very subtle and may not involve drastic alteration in the genetic background. It is therefore possible to accumulate somaclonally induced advantages in already improved cultivar; (iii) somaclonal variation occurs for traits of both nuclear and cytoplasmic origin. The variation of cytoplasmic genes obtained by this method is a distinct advantage; and (iv) in wide crosses, somaclonals provide a mechanism of gene introgression. Immature embryos of the wide cross can be callused and plants with the introgressed desired gene (or gene complex) are selected among the regenerants or their progenies.

Somaclonal variation has been observed in all cases where it has been looked for. It has been documented in a wide variety of plant species and occurs irrespective of the type of explant cultured.

Interest in somaclonal variation is relatively recent (Bhaskaran, 1985) and therefore the mechanism of its causation has not yet been firmly established. The following possibilities have been considered as possible mechanism for the generation of somaclonal variation.

1) The pre-existing genetic variation in the explant tissue,

2) The spontaneous mutations which can accumulate during the many division cycles that cells of the explant go through before differentiating into an *in vitro* plant. The recessive mutations will naturally require a method by which they

can express even in diploid cells. Somatic crossing over followed by segregation is a likely mechanism for the creation of homozygosity and thus phenotypic expression of the recessives.

3) Numerical and structural changes in chromosomes during *in vitro* growth,

4) Intracellular mutagenic agents produced during *in vitro* growth.

5) Activation of transposable elements. Transposable elements, sometimes also referred to as jumping genes, are genetic entities which have the property of spontaneously changing their position within the genome. The locus at which they get integrated is mutated.

Already, somaclonal variation has been processed into commercial cultivars of sugar cane in Hawaii and in India. The characteristics improved by this procedure include yield and disease resistance. Practical advantages from somaclonal variation are expected when this technique is fully integrated with the conventional plant breeding procedures.

6. Micropropagation

Several situations in agriculture and horticulture require procedures of rapid multiplication of elite genotypes vegetatively. These include: (i) when the requirements of multiplication of a genotype are very large and the conventional methods of vegetative propagation are either not available or are not efficient, (ii) when, as in potato, the cost of the seed potato forms the major part of production cost, (iii) when it is desired to selectively propagate a particular sex as in the case of papaya, (iv) when the use of conventional methods of breeding is limited by long generation time and long period of juvenility, as is obtained in plantation crops and in forest trees.

Micropropagation requires *in vitro* culture. Two methods of exploitation of *in vitro* culture for large-scale multiplication are: (a) multiple shootlet production, and (b) somatic embryogenesis.

a) *Multiple shootlet* production relies on differentiating large number of shoot primordia from an explant without intervening callusing. The regenerated shootlets are rooted to produce plantlets. This procedure is being extensively practiced for potato propagation in Korea, Vietnam and other countries in the Far East. Tissue culture multiplication is also being used for generating material for commercial plantation of oil palm in Malaysia. In India, procedures have been standardized for *in vitro* multiplication of both oil palm and coconut, even though improvements are yet required for field application of these techniques. However, in cardomom and in orchids commercial tissue culture multiplication is being done.

b) *Somatic embryogenesis* technique, as a method of micropropagation, has the advantage of cost effectiveness because production cost per unit can be kept at affordable level. Somatic "embryos" are structures developing from somatic cells which resemble the sexual embryos in morphology. They differentiate shoots and roots simultaneously. Protocols have been worked out by which somatic embryos can be produced in large number in liquid cultures. Culturing in liquid medium also allows easy handling. Recent investigations have shown that the harvested somatic embryos can be used as 'artificial seeds' after induction of dormancy by treatment with abscisic acid and coating them with synthetic material (*e.g.* polyethylene oxide and calcium alginate). Artificial seeds with normal germinability have been produced at the experimental scale in carrot, hybrid alfalfa and celery.

7. Genetic engineering and plant cell transformation

Genetic engineering simulates genetic recombination at the DNA level outside the cell. It involves isolation of a DNA stretch corresponding to a particular function of a donor and hooking it to a vector for the purpose of transference. It can also involve, when the amino acid sequence of the protein product of a gene is known, synthesizing a gene artificially on the basis of genetic code dictionary and transferring it to the desired recipient.

The following steps of the recombinant DNA technology, also referred to as genetic engineering, are important.

1) *Gene identification:* There are several methods of isolating a particular gene (Figs. 2, 3 and 4). The first one is applicable when the gene produces a large enough amount of mRNA that can be isolated. The mRNA is copied, by reverse transcriptase, into corresponding cDNA which in turn is used as a probe to fish out the gene from genomic libraries. Genomic library refers to the pool of DNA fragments of a genome produced by the action of restriction enzymes and maintained as clones in a suitable host, usually *E. coli*. In the second method, the protein product of the gene is used as the starting material to artificially synthesize DNA segments based on amino acid sequence of the protein. These DNA segments can be used as probes for identifying the gene from genomic libraries. These two methods have been most widely used. Other methods of gene identification are chromosome walking, as practiced in *Drosophila,* and use of transposable or mobile elements in plants.

2) *Gene transfer:* Once the desired gene has been identified and isolated, it needs to be transferred to the recipient. Two major avenues are currently used for this purpose:

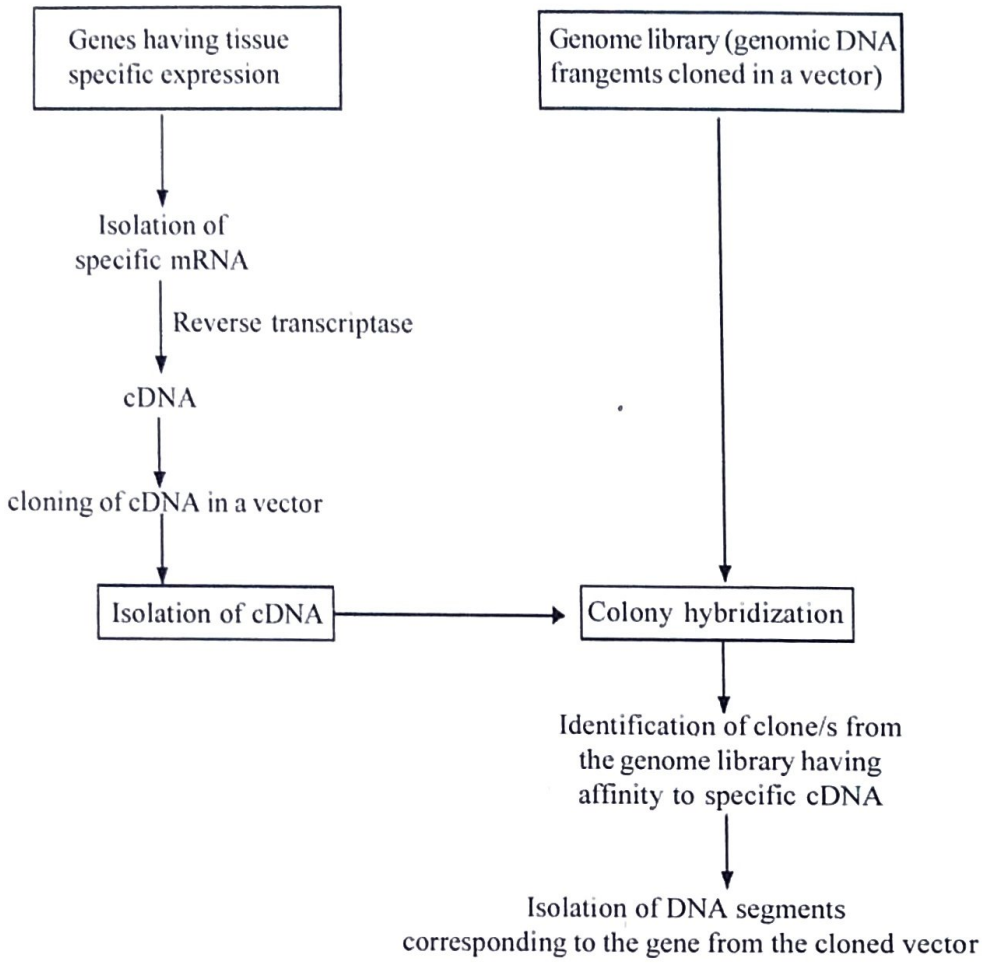
Genes having tissue specific expression
Genome library (genomic DNA frangemts cloned in a vector)
Isolation of specific mRNA
Reverse transcriptase
cDNA
cloning of cDNA in a vector
Isolation of cDNA
Colony hybridization
Identification of clone/s from the genome library having affinity to specific cDNA
Isolation of DNA segments corresponding to the gene from the cloned vector

Fig. 2. Isolation procedure for genes through mRNA

a) vector mediated transfer which uses the agency of plasmids and viral vectors. Among the plasmids, T_1 of *Agrobacterium tumifaciens* is the most extensively used and the most promising one. It affects gene transfer in dicotyledenous species with high frequency. Even though its effectiveness has also been demonstrated for some monocots, *e.g. Narsisus,* further developments are needed for making Ti plasmid a workable tool for gene transfer in monocots. Attempts are also underway to develop plant viruses, like cauliflower mosaic virus (CaMV), as vectors for gene transfer.

b) plant transformation: this procedure aims at direct introduction of DNA segment into the plant cell. Protoplasts and pollen are considered suitable material for DNA introduction. Among the methods tried for achieving this are co-cultivation (incubating the recipient cells with purified or gene enriched DNA), electroporation (carried out by applying high electric potential for microseconds to change the porosity of the cells to allow intake of DNA molecules) and microinjection (using very fine glass needles to inject the desired transforming DNA directly into the nucleus).

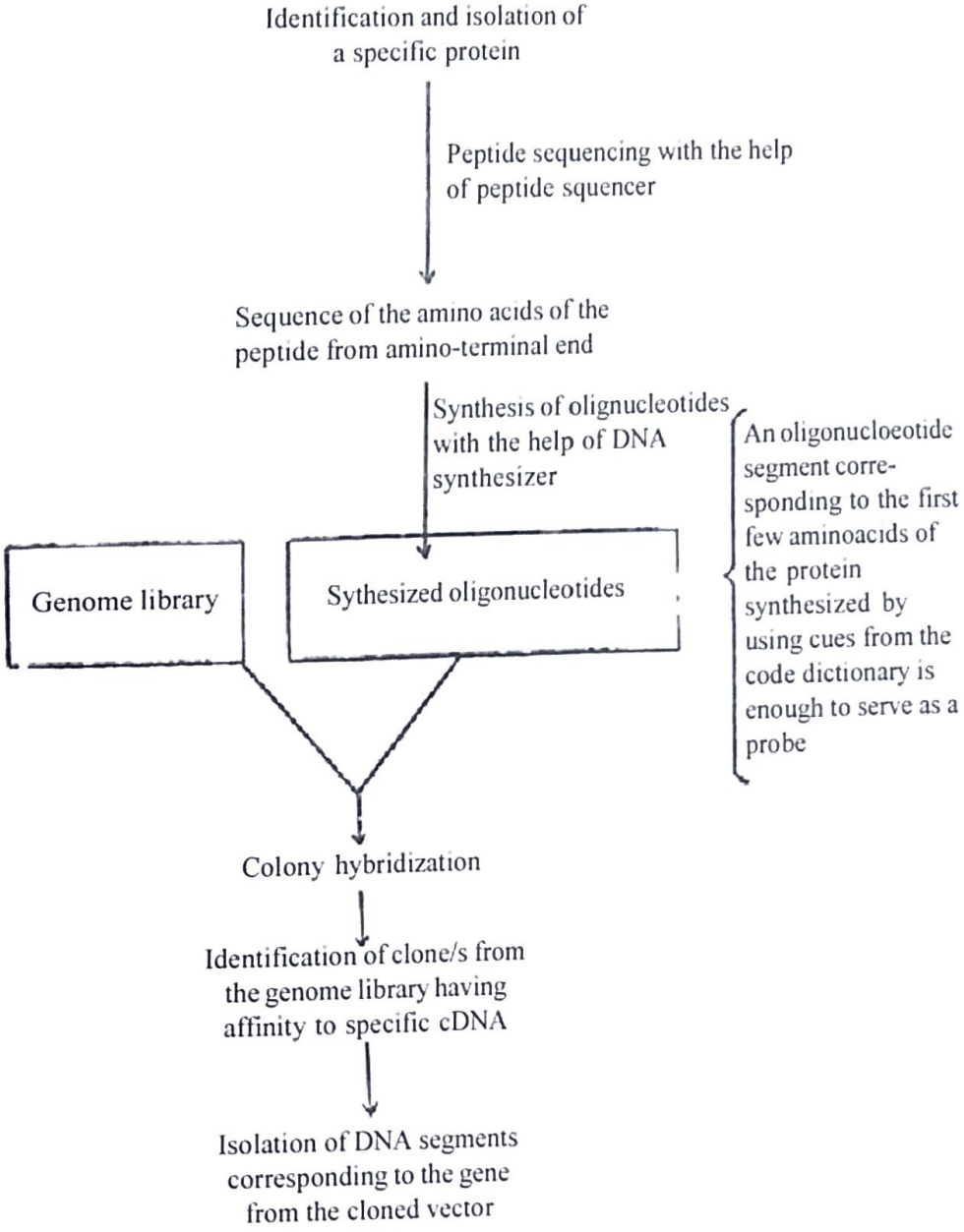
Identification and isolation of a specific protein
Peptide sequencing with the help of peptide squencer
Sequence of the amino acids of the peptide from amino-terminal end
Synthesis of olignucleotides with the help of DNA synthesizer
An oligonucloeotide segment corresponding to the first few aminoacids of the protein synthesized by using cues from the code dictionary is enough to serve as a probe
Genome library
Sythesized oligonucleotides
Colony hybridization
Identification of clone/s from the genome library having affinity to specific cDNA
Isolation of DNA segments corresponding to the gene from the cloned vector

Fig. 3. Isolation of genes through protein route.

3) *Gene integration and expression:* For a change to be heritable, stable integration of the donor DNA into the recipient genome is essential. The basic mechanism for this integration is through breakage and reunion following pairing of homologous regions. Even after integration, a foreign gene will express only when it receives relevant signals. When integrated segment is complete (structural plus regulatory elements), the transferred genes are normally expressed. If complete regulatory signals are not available in the donor DNA, the genes will express only when brought under control of the host regulatory elements.

Genetic engineering has tremendous potentials for improving crop plants and has opened up avenues of genetic manipulation which were unthinkable even a couple of decades ago. The complexity of organization of the genetic material in eukaryotes, inadequate knowledge of gene expression and the wide gap in our understanding of the biochemical pathways between the initial steps of gene action and realization of the phenotypes necessitates considerable more work before recombinant DNA technology finds general application for field crop improvement. It is also important to keep in mind that the major contribution of this new technology will be to reach the stage of a stabilized recombinant in the conventional plant breeding procedure. For attaining the maximum gains from this procedure, therefore, conventional plant breeding will still retain relevance when all the methodologies of genetic engineering are perfected. An integrated approach involving the techniques of new biology and the time tested procedures of agricultural improvement would be the most profitable option for the future.

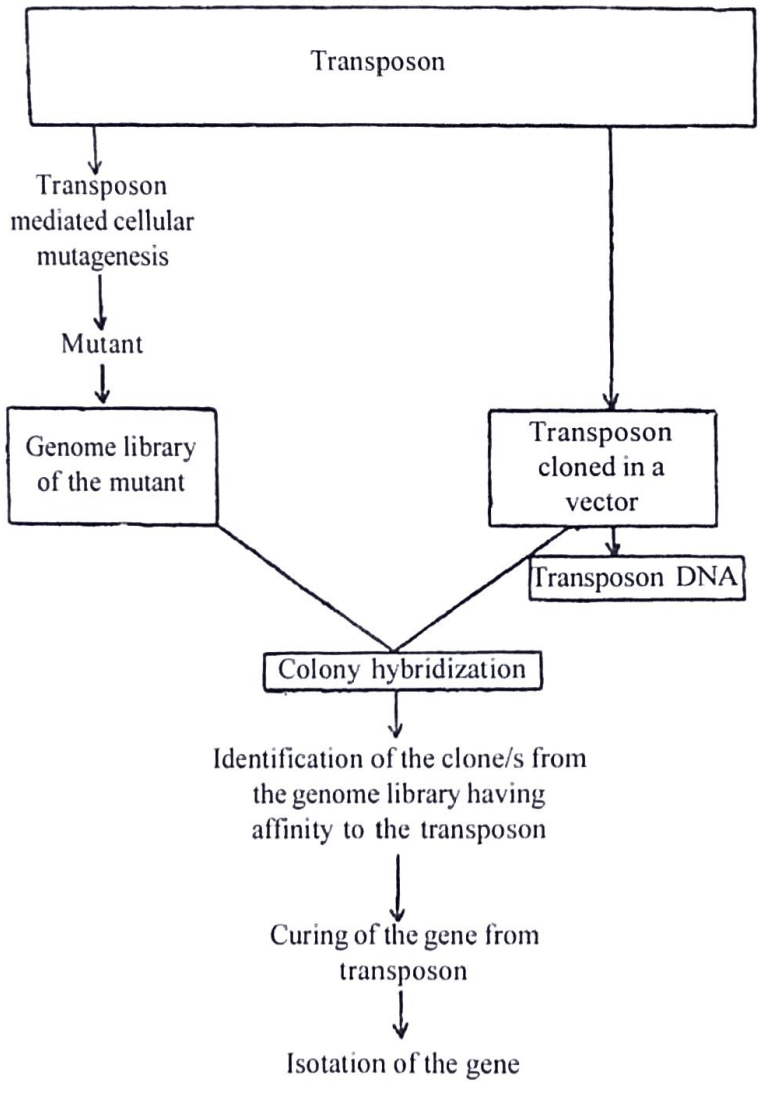
Transposon
Transposon mediated cellular mutagenesis
Mutant
Genome library of the mutant
Transposon cloned in a vector
Transposon DNA
Colony hybridization
Identification of the clone/s from the genome library having affinity to the transposon
Curing of the gene from transposon
Isotation of the gene

Fig. 4. Use of transposon mutagenesis for identification and isolation of a gene

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